

RELATIONSHIPS BETWEEN WEIGHT, HOMA IR, LEPTIN, ADIPONECTIN AND  
INTERLEUKIN-6, BEFORE AND AFTER A CALORIE RESTRICTED DIET  
INTERVENTION, AND IN A 6-8 MONTH POST DIET PERIOD, IN OVERWEIGHT AND  
OBESE INDIVIDUALS AT RISK FOR TYPE 2 DIABETES

By

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To the faculty of Washington State University:

The members of the Committee appointed to examine the thesis of  
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Chair

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Abstract

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Obesity is rapidly becoming a worldwide health problem with profound effects on health care costs and individual health. The economic cost of obesity reaches billions of dollars yearly in the United States and continues to rise. Understanding the role of adipose tissue as an endocrine organ and the underlying relationships and interactions of adipose derived cytokines in insulin resistance, type 2 diabetes and the metabolic syndrome is the subject of ongoing research. Serum measurements of cytokines and insulin sensitivity have been noted to change in weight loss induced by calorie restriction.

The purpose of this repeated measures design study was to evaluate the effects of a 28 day calorie restricted diet intervention on weight, serum measures of insulin sensitivity (HOMA IR), and the serum cytokines, leptin, adiponectin and interleukin-6 (IL-6). Data were collected at baseline T1, at the end of a calorie restricted diet intervention T2, and in a six to eight month post diet period T3, in a group of overweight or obese individuals ( $BMI \geq 26 \text{ kg/m}^2$ ) at risk for type 2 diabetes.

Nine subjects, out of the larger parent pilot study (n = 20), provided data for each of the three time comparisons. Serum measures of leptin, adiponectin and IL-6 were measured using ELISA analysis. Significant decreases in T1 to T2 comparisons were found for weight, leptin, HOMA IR and adiponectin. IL-6 decreased but the change did not reach statistical significance. Nearly all the subjects regained weight in the post diet period, and serum levels of HOMA IR and the cytokines returned to baseline. At baseline, adiponectin and weight, and adiponectin and HOMA IR, demonstrated significant negative correlations. Change scores for the T1 to T2 time period were significantly correlated for adiponectin and weight. No significant change scores correlations were noted for the T2 to T3 period.

Results of this study suggest that in a small cohort of individuals at risk for developing diabetes, short term calorie restriction achieved significant weight loss and changes in HOMA IR and some cytokines, but the changes were not sustained. Subjects in this study, for the most part, regained weight and HOMA IR and measured cytokines returned to baseline. Therefore, short-term calorie restricted dieting cannot be recommended as a strategy for improving risk factors for developing type 2 diabetes in this population.

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## CHAPTER 1

### INTRODUCTION

“Obesity is by far the most common nutritional disorder in Europe and in North America and is sharply increasing in other parts of the world as well. It is even possible to foresee a time when obesity overtakes malnutrition as a major worldwide health burden” (Tappy & D'Alessio, 2006, p 455).

The 1976-1980 National Health and Nutrition Examination Survey (NHANES) estimated that 15% of the adult population in the US was obese (Centers for Disease Control [CDC], 2007). In the 2003-2004 NHANES survey, 32.9% of the US adult population was obese, while the total of overweight and obese adults reached 66.2% of the adult US population (CDC, 2007). The economic cost of overweight and obesity in the United States reached \$117 billion in 2001 (National Institute of Diabetes and Digestive and Kidney Disease [NIDDK], 2007).

Body mass index (BMI) is an index of an individual's weight adjusted for height and is used to define individuals as overweight or obese (NIDDK, 2007). BMI is calculated as weight in kilograms divided by height in meters squared ( $\text{kg}/\text{m}^2$ ) (CDC, 2007; NIDDK, 2007). Among adults (age 20 and older), overweight is defined as a BMI of 25 to 29.9  $\text{kg}/\text{m}^2$ , and obese is defined as a BMI of  $\geq 30 \text{ kg}/\text{m}^2$  (NIDDK, 2007; CDC, 2007).

Obesity and associated risk factors of insulin resistance, dyslipidemia, and high blood pressure are known as metabolic syndrome (Reaven, Lithel, & Landsberg, 1996). Because obesity is associated with insulin resistance and type 2 diabetes, much focus has been on weight reduction strategies. The underlying assumption is that weight loss will improve insulin

resistance. However, the relationship between obesity and insulin resistance in individuals predisposed to type 2 diabetes is not clearly defined (Tappy & D'Alessio, 2006).

Insulin resistance is associated with body fat, particularly intra-abdominal fat, and body composition (Kahn, 2003; Tappy & D'Alessio, 2006). Central fat is more related to insulin resistance than peripheral fat, and visceral fat is more related to insulin resistance than subcutaneous abdominal fat (Bonora, 2000).

The hallmark of insulin resistance is hyperinsulinemia, and is the body's attempt to overcome decreased insulin sensitivity in tissue in the face of hyperglycemia (Tappy & D'Alessio, 2004). Normal glucose metabolism has been described as a kind of feedback loop in which elevated plasma glucose levels stimulate insulin secretion, which in turn stimulates glucose uptake into cells, while inhibiting further glucose production (Gerich, 1993).

Type 2 diabetes is the result of a combination of impaired insulin release and insulin resistance (D'Alessio, Le, & Tappy, 2005; Schuman, 2000). The eventual result is that  $\beta$  cells of the pancreas fail to increase secretion of insulin in the face of decreased insulin sensitivity (D'Alessio et al., 2005). By the time hyperglycemia appears, tissue insulin sensitivity and  $\beta$  cell function have already decreased (Kahn, 2003).

Although there has been considerable evidence linking obesity to insulin resistance, the underlying mechanisms of this association are debated (Tappy & D'Alessio, 2006). Some have argued that the mechanism linking obesity and insulin resistance is circulating free fatty acids. The thought is that adipose tissue lipolysis, in persons with increased fat mass, results in increased circulating free fatty acids, which in turn are responsible for impaired glucose uptake in tissue (Felber, 1992). More recent studies suggest the mechanism may be more complex, and

that an accumulation of lipid derived intracellular metabolites, through increased delivery or decreased metabolism, may induce insulin resistance, and subsequently decrease glucose transport into cells (Schuman, 2000).

Others suggest that “ectopic” lipids may be contributing to insulin resistance. The thought is that as obesity progresses, lipids are deposited in other non-adipose organs and tissues, such as the liver, skeletal muscle, pancreas, and cardiac tissue. The resulting lipid deposition leads to lipotoxicity, impairing these organs and tissues, and subsequently results in tissue insulin resistance, impaired insulin  $\beta$ -cell secretion, and finally type 2 diabetes (Molavi, Rasouli, & Kern, 2006; Goodpaster, He, Watkins, & Kelley, 2001; Greco, et al., 2002).

A number of pro-inflammatory cytokines or endocrine factors, secreted by adipose tissue, may contribute to the underlying mechanisms linking obesity to insulin resistance (Alihaud, 2000; Trayhurn & Wood, 2004). The terms “adipokines” or “adipocytokine” are used to denote those substances that are primarily produced by adipocytes (Lago, Dieguez, Gomez-Reino, & Gualillo, 2007; Fantuzzi, 2005), such as adiponectin and leptin, whereas the terms “cytokine” or “chemokine” refer to substances which are produced only in part by adipose tissue (Fantuzzi, 2005). Interleukin 6 (IL-6) and tissue necrosis factor alpha (TNF- $\alpha$ ) are examples of cytokines produced in part by adipose tissue but not strictly considered an adipocytokine (Tilg & Moschen, 2006). For the purposes of this study, the term “cytokine” will be used when referring to adiponectin, leptin and IL-6.

With obesity, it is hypothesized that pro-inflammatory cytokines, such as leptin, and IL-6 are released by adipose tissue and have an inflammatory effect in the tissue milieu (Vendrell et al., 2004). Their release may somehow mediate whole body insulin resistance (Tappy &

D'Alessio, 2006). Reduction in fat mass decreases serum levels of inflammatory cytokines, such as leptin and IL-6, with the subsequent proinflammatory environment associated with obesity attenuated (Vendrell et al., 2004). The reduction of these metabolic noxious stimuli may then allow correction of glucose homeostasis (Tappy & D'Alessio, 2006).

Adiponectin, one of the more abundant secretory products of adipose tissue, is thought to improve insulin sensitivity and inhibit vascular inflammation (Scherer, Williams, Fogliano, Baldini, & Lodish, 1995; Lyon, Law, & Hsueh, 2003). In obese subjects serum adiponectin is low, but increases with weight loss (Vendrell et al., 2004; Arita et al., 1999; Yang et al., 2001).

Some of the relationships between weight loss/calorie restriction and weight gain to obesity, IL-6, adiponectin, leptin, and insulin resistance are summarized below.

Table 1. Relationships between Obesity, Insulin Resistance and Cytokines in Weight Loss/Gain.

	Weight Loss/Calorie Restriction	Weight Gain
Obesity	Decreased	Increased
IL-6	Decreased	Increased
Adiponectin	Increased	Decreased
Leptin	Decreased	Increased
Insulin Resistance	Decreased	Increased

Although the mechanisms linking body fat, insulin resistance and lipids are not clearly defined, it is recognized that weight loss increases insulin action and improves or even alters glucose homeostasis (Tappy & D'Alessio, 2006). Although weight loss has been shown to improve insulin resistance, this may be attributed to more than simply a decrease of fat mass. The improvement may also be attributed to caloric restriction (Tappy & D'Alessio, 2006).

Reducing calorie intake has been shown to improve glycemic control more rapidly than weight loss, and before changes in body composition occur (Kelly et al., 1993; Felber et al., 1981). This has also been noted after gastric bypass surgery, where glycemia was improved before changes in body weight (Greenway, Greenway, III, & Klein, 2002; Buchwald et al., 2004). By contrast, liposuction removal of large amounts of fat did not improve insulin sensitivity (Klein et al., 2004), underscoring the thought that metabolic consequences of excess body fat are dependent on the location of fat deposits in the body, and that factors other than just total body fat are in some way linked to insulin sensitivity (Tappy & D'Alessio, 2006).

Although surgery may be an option for treatment of obesity, there are potential side effects and costs that do not make it a first line choice. Behavioral strategies developed from social learning have been the most studied non-surgical weight loss interventions, and are recommended as a first line treatment for any weight loss effort (Levy, Finch, Crowell, Talley, & Jeffery, 2007), but long term weight loss results are discouraging.

The major problem many people face after losing weight is that they are unable to maintain the weight loss long term (Wallner et al., 2004; Kayman, Bruvold, & Stern, 1990; Gorin, Phelan, Wing, & Hill, 2004). The cycling of weight loss and weight gain has been shown to promote abdominal adiposity and may contribute to long term health risks (Rodin, Radke-Sharpe, Rebuffe-Scrive, & Greenwood, 1990; Jefferey, 1996; Truesdale, Stevens, Schreiner, Loria, & Cai, 2006; Hamm, Shekelle, & Stamler, 1989; Lissner et al., 1991; Lee & Paffenbarger, 1992). With weight regain and return to obesity, it has been observed that fat is recovered at a faster rate than lean tissue (Dulloo, Jacquet, & Montani, 2002), and redistributed to a greater



degree to upper body compartments, which in turn strengthens the risk for metabolic syndrome (Wallner et al, 2004).

#### Statement of Problem

The connections between obesity and insulin resistance are not fully understood and continue to be the subject of much study. There is limited research looking at the effects of weight loss and calorie restriction on insulin sensitivity, and various cytokines, such as leptin, adiponectin, and IL-6, in a 6-8 month post-diet period, compared to measures taken before and after a calorie restricted dietary intervention.

Most studies note improvement of serum markers after caloric restriction or weight loss, perhaps because markers are measured in a calorie restricted or weight loss state. Few differentiate between weight loss and caloric restriction, and even fewer have data measured in a 6-8 month post diet period, after a weight loss or caloric restriction intervention. There is limited research in humans measuring insulin, adiponectin, leptin and IL-6 in a 6-8 month post-diet period following caloric restriction and weight loss.

#### Statement of the Purpose

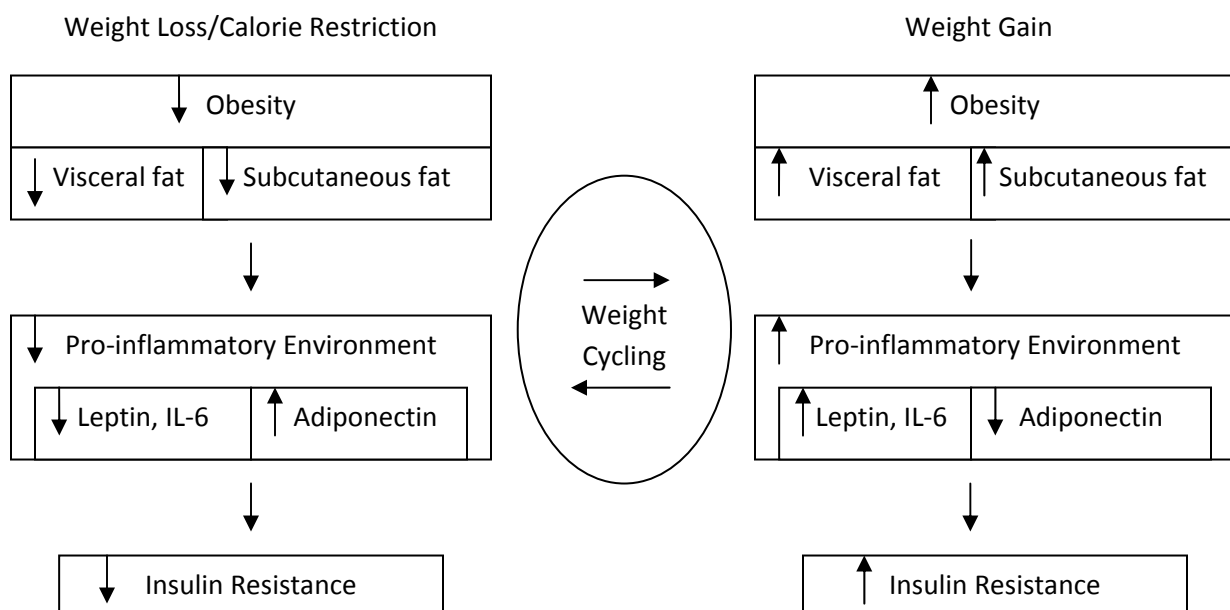
The purpose of this study was to examine changes in serum concentrations of insulin, leptin, adiponectin, and IL-6 before, and after a calorie restriction intervention, and in a 6-8 month post diet period, in a convenience sample of 20 overweight and obese individuals (BMI  $\geq$  26 kg/m<sup>2</sup>) with a family history of type 2 diabetes.

#### Conceptual Framework

It is helpful to frame obesity, caloric restriction, weight loss, and the various metabolic abnormalities of insulin resistance and cytokines in the framework of systems theory. Systems

theory implies that “all the components of a system are related, and any changes in one component will affect all the others” (Zikovic & German, 2007, p 240). A leading expert on metabolic syndrome, Scott Grundy, states “one question that is repeatedly asked about the metabolic syndrome is whether its whole is more than its parts...whether the syndrome confers a greater risk ...than does its component risk factors” (Grundy, 2007, p 241), an idea of *emergence*, implying “characteristics exhibited on the level of the whole...but not the components in isolation” (Wikipedia, 2007, p 6). The underlying connections between obesity and insulin resistance are unknown, as is the pathological sequence for type 2 diabetes, which involves many different elements acting in concert to cause the disease (Leahy, 2005). Figure 1 illustrates possible connections between obesity, insulin resistance, and cytokines in relationship to weight loss/calorie restriction and weight gain.

Figure 1. Possible Connections between Obesity, Insulin Resistance, Leptin, IL-6, Adiponectin in Weight Loss/Calorie Restriction and Weight Gain.



## Review of the Literature

### Definition and Measurement Issues

#### *Metabolic Syndrome*

Metabolic syndrome encompasses various clinical and biochemical abnormalities that are associated with insulin resistance, including glucose intolerance, central obesity, lipid abnormalities and hypertension (Cefalu, 2000). Although these associations are noted with insulin resistance, the cause and effect is difficult to establish. In addition, there is disagreement as to what the syndrome should be called, and diagnostic criteria vary with the group of experts doing the defining. To illustrate, Table 2 shows the difference between the World Health Organization (WHO), the 2001 National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III), and the American Heart Association (AHA) criteria (Alberti & Zimmet, 1998; National Cholesterol Education Program (NCEP) Adult Treatment Panel III final report, 2002; Grundy et al., 2005)

The American College of Endocrinology (ACE), on the other hand, states that a precise definition of metabolic syndrome is neither possible nor valuable. They argue that obesity should not be included in the syndrome, because obesity is a cause of, rather than a result of insulin resistance. In addition, there is some disagreement over whether people with diabetes should be included in the definition of insulin resistance syndrome (Einhorn et al., 2003; Shaw & Zimmet, 2003).

#### *Measurement of Insulin Resistance*

Insulin resistance is associated with the risk factors of metabolic syndrome. It is defined as “a clinical state in which a normal or elevated insulin level produces an impaired biological

Table 2. Criteria for Clinical Diagnosis of Metabolic Syndrome.

	WHO	ATP III	AHA
Clinical Measure	Insulin resistance plus two risk factors	Three of five risk factors	Three of five risk factors
Waist Circumference		≥ 102 cm men ≥ 88 cm women	≥ 102 cm men ≥ 88 cm women
BMI	BMI > 30 kg/m <sup>2</sup>		
Triglycerides	≥ 150 mg/dL	≥ 150 mg/dL	≥ 150 mg/dL
HDL-C	< 35 mg/dL in men < 39 mg/dL in women	< 40 mg/dL in men < 50 mg/dL in women	< 40 mg/dL in men < 50 mg/dL in women
Blood Pressure	≥ 140/90 mm Hg	≥ 130/85 mm Hg	≥ 130/85 mm Hg
Glucose	IGT, IFG or T2D	Fasting > 110 mg/dL (IFG)	Fasting ≥ 100 mg/dL (IFG)
Insulin Resistance	Euglycemic clamp	No	No
Microalbumuria	Yes	No	No

Abbreviations: WHO=World Health Organization; ATP III=Adult Treatment Panel III; AHA= American Heart Association; BMI= Body mass index; IGT= Impaired Glucose Tolerance; IFG= Impaired Fasting Glucose; T2D= Type 2 Diabetes (Alberti & Zimmet, 1998; National Cholesterol Education Program (NCEP) Adult Treatment Panel III final report, 2002; Grundy et al., 2005; Lab Tests Online, 2008).

response” (Cefalu, 2000, p. 57). An individual will increase insulin secretion to compensate for insulin resistance in tissues such as fat, muscle and liver to maintain glucose homeostasis.

Eventually as  $\beta$  cell function decreases, resulting in less insulin being produced, blood glucose levels rise, which in turn results in the diagnosis of type 2 diabetes (Cefalu, 2000). By the time hyperglycemia appears, years of insulin resistance may have gone unnoticed. The WHO criteria

list the euglycemic clamp as a diagnostic of insulin resistance for the metabolic syndrome (Grundy et al., 2005).

There are several methods of measuring insulin resistance in research studies including the euglycemic clamp, the homeostasis model assessment of insulin resistance (HOMA IR), and the insulin sensitivity index (ISI).

#### *Euglycemic Clamp*

Briefly explained, the euglycemic clamp is a measure of tissue sensitivity to exogenous insulin. The technique involves a primary infusion of insulin to raise plasma concentrations to 100  $\mu\text{U}/\text{mL}$ . Plasma glucose is held constant at basal levels by a variable glucose infusion. The result is a steady state of euglycemia in which the glucose infusion rate equals glucose uptake by all the tissues of the body (DeFronzo, Tobin, & Andres, 1979).

The euglycemic clamp is the ideal research method to evaluate impaired insulin sensitivity to glucose homeostasis, and considered the gold standard to which other measurements of insulin sensitivity are compared (Matsuda & DeFronzo, 1997). However, the method is cumbersome and not practically available to all researchers.

#### *Homeostasis Model Assessment of Insulin Resistance*

Another method of measuring insulin resistance used in research studies is the homeostasis model assessment of insulin resistance (HOMA IR), proposed by Mathews and colleagues, to measure insulin resistance (Mathews, Hosker, Rudenski, Naylor, Treacher, Turner, 1985). The formula for HOMA IR is the product of fasting plasma insulin (FIRI)  $\mu\text{U}/\text{ml}$  x fasting plasma glucose (FPG)  $\text{mmol}/\text{l}$  divided by 22.5 (Mathews et al., 1985).

The estimate of insulin resistance obtained by HOMA IR, correlates with estimates by euglycemic clamp ( $r = 0.88$ ,  $p < 0.0001$ ) (Mathews et al., 1985), and ( $r = 0.69$ ,  $p < 0.0001$ ) (Matsuda & DeFronzo, 1999). The model presumes that hepatic and peripheral insulin sensitivity are equivalent (Matsuda & DeFronzo, 1999). HOMA IR is the method of measuring insulin resistance used in some of the subsequent articles discussed further in the literature review.

### *Insulin Sensitivity Index*

The insulin sensitivity index (ISI) was developed by Matsuda and DeFronzo (1999), using oral glucose tolerance testing (OGTT) data. The formula for the index ( $10,000/\text{square root of} [\text{fasting glucose} \times \text{fasting insulin}] \times [\text{mean glucose} \times \text{mean insulin during OGTT}]$ ) is highly correlated ( $r = 0.73$ ,  $p < 0.0001$ ) to euglycemic insulin clamp, and is a composite of hepatic and peripheral tissue sensitivity to insulin (Matsuda & DeFronzo, 1999).

The OGTT is used in many metabolic and epidemiological studies. The ISI, one of several methods of measuring insulin resistance, is a more practically available method to measure insulin sensitivity using OGTT data, following the formula outlined above (Matsuda & DeFronzo, 1999).

### *Measurement of Obesity*

Obesity is an excess of adipose tissue (Molavi et al., 2006), and is associated with insulin resistance and metabolic syndrome. It can be measured using body mass index (BMI), but BMI does not give any indication of distribution of body fat. Upper body obesity strengthens the risk for metabolic syndrome (Wallner et al., 2004).

### *Body Mass Index*

Body mass index (BMI) is an index of an individual's weight adjusted for height and is used to define individuals as overweight or obese (NIDDK, 2007). BMI is calculated as weight in kilograms divided by height in meters squared ( $\text{kg}/\text{m}^2$ ) (CDC, 2007; NIDDK, 2007). Among adults (age 20 and older), overweight is defined as a BMI of 25 to 29.9  $\text{kg}/\text{m}^2$ , and obese is defined as a BMI of  $\geq 30 \text{ kg}/\text{m}^2$  (NIDDK, 2007; CDC, 2007).

### *Waist Circumference*

Waist circumference measurement is a more practical clinical measurement of abdominal obesity. The ATP III defines obesity using waist circumference of  $\geq 102 \text{ cm}$  ( $\geq 40$  inches) for men and  $\geq 88 \text{ cm}$  ( $\geq 35$  inches) for women. There may be differences among ethnic groups (Grundy et al., 2005). Waist circumference is measured by locating the top of the right iliac crest and then placing a tape measure in a horizontal plane around the abdomen at the level of the iliac crest. The tape measure should be snug but not compressing the skin and parallel to the floor. Measurement is made at the end of expiration (Grundy et al., 2005).

Although waist circumference is an indicator of central fat distribution, it cannot give any information about the proportions of subcutaneous abdominal adipose tissue compared to visceral adipose tissue (Bonora, 2000). Computerized tomography (CT) and magnetic resonance imaging (MRI) are more sensitive measures to distinguish central subcutaneous and visceral adipose tissue distribution (Bonora, 2000).

### *Waist to Hip Ratio*

Waist to hip ratio (WHR) is another method to measure obesity and is more consistently associated with coronary heart disease than body mass index in both men and women (Canoy

et al., 2007). While the subject is standing, the waist circumference and the hip circumference are measured. The waist is the largest abdominal circumference midway between the costal margin and the iliac crest. The hip is the largest circumference just below the iliac crest (Moyad, 2004). A WHR in women greater than 0.80, and in men greater than 0.90, is a fairly accurate predictor of an increased risk of obesity-related conditions, which is independent of BMI (Gray & Fujioka, 1991; Solomon & Manson, 1997).

### The Role of Adipose Tissue in Insulin Resistance

#### *Visceral versus Subcutaneous Fat*

Visceral fat is a strong predictor of insulin resistance in obese subjects but not in normal weight individuals (Bonora, 2000). Abdominal subcutaneous fat has higher metabolic activity than lower body subcutaneous fat (Cefalu, 2000). Visceral abdominal fat is inversely correlated with insulin sensitivity (Bonora et al., 1992). After analyzing 15 papers reporting the relationship between abdominal fat and insulin resistance between 1991 and 1998, Bonoro (2000) concludes, with the exception of two papers, that central fat has a higher degree of association with insulin resistance than peripheral fat, and visceral fat is more related to insulin resistance than subcutaneous fat (Bonora, 2000).

Hence, visceral fat may be more important than overall fat mass in the pathophysiology of insulin resistance. In studies with rats, caloric restriction has been shown to reverse hepatic insulin sensitivity (measured by insulin clamp) in aging rats to levels of younger rats by decreasing visceral fat, while peripheral insulin sensitivity remained unchanged (Barzilai, Bannerjee, Hawkins, Chen, & Rosetti, 1998). In other studies, excess visceral fat is associated with higher plasma glucose and insulin, independent of overall obesity (Wajchenberg, 2000),



and visceral fat adiposity is noted to have more insulin resistance than subcutaneous fat adiposity (Matsuzawa, 2006). By contrast, Goodpaster and coworkers (1997) were able to show that both visceral adiposity and subcutaneous adiposity were independently related to insulin resistance (Goodpaster, Thaete, Simoneau, & Kelley, 1997).

### *The Cytokines*

Up to the early 1990s, adipose tissue was thought simply to be an energy storage organ, but with the continued discovery of various cytokines, adipose tissue is now being viewed as a highly active endocrine organ with various metabolic activities (Vendrell et al., 2004), even the largest endocrine organ of the human body (Bulcao, et al, 2006). Adiponectin, leptin, IL-6, TNF- $\alpha$ , resistin, adiponin and visfatin are among the many adipocytokines that have been identified and are the continued subject of much study in their relationship to obesity, insulin resistance, weight loss, and diabetes.

### *Leptin*

Leptin was discovered as a hormone produced by adipocytes in 1994 (Zhang et al., 1994). Leptin is a 16kDa protein that is coded by an obese (*ob*) gene (Fantuzzi, 2005; Lago et al., 2007; La Cava & Matarese, 2004). Leptin is directly correlated with body weight and fat mass (Barzilai & Gupta, 1999; Lago et al., 2007). Leptin secretion has been shown to be 2-3 times higher in subcutaneous fat as compared to omental fat tissue (Van Harmelen et al., 1998). Serum levels are 2-3 times higher in women than men with similar body fat mass (La Cava & Matarese, 2004; Tilg & Moschen, 2006; Lago et al., 2007).

Although mainly produced by adipocytes, leptin may also act as a pro-inflammatory cytokine (Tilg & Moschen, 2006), though the role of leptin in inflammation is not fully

understood. Leptin may play a role in inflammatory and autoimmune diseases, while at the same time protect against infections in an infectious disease state (Fantuzzi, 2005; Tilg & Moschen, 2006).

Leptin is noted to regulate food intake and basal metabolism (La Cava & Matarese, 2004). Its role in weight regulation normally may involve afferent signaling in a negative feedback loop to the hypothalamus. As leptin levels rise, the result is a negative energy balance (energy expenditure > food intake). Decreasing leptin levels result in a positive energy balance (food intake > energy expenditure) (Friedman, 2002). Leptin then functions like a signaling factor to the brain, similar to an endocrine hormonal energy balance sensor (Bulcao, Ferreira, Giuffrida, & Ribeiro-Filho, 2006; Badman & Flier, 2005). Leptin levels have been noted to drop in starvation, which is understood to be adaptive to decreased energy stores, as well as a response in part to the hormonal mechanisms described above. Weight gain causes a different hormonal response. Increasing levels of leptin lead to a state of negative energy balance (Friedman, 2002).

The high correlation of body fat content and plasma leptin levels is suggested by some researchers to reflect leptin “resistance” or “insensitivity” (Friedman, 2002). This can be thought of much in the same way increased insulin levels reflect insulin resistance (Heymsfield et al., 1999; Hukshorn et al., 2002). A low or normal plasma leptin level in the context of obesity would suggest decreased production of leptin, much like decreased insulin is noted in the pathway to diabetes (Friedman, 2002).

Generally, obese humans have high leptin levels, but five to ten percent of obese humans have low levels of leptin (Maffei et al., 1995; Ravussin et al., 1997). Pima Indians, for

instance, are noted to have low plasma leptin and are more predisposed to weight gain, obesity and diabetes (Ravussin et al., 1997). In the case of the Pima Indians, low leptin levels are thought to be an evolutionary trait over years in response to periods of starvation and survival, but not so positive in our present affluent society (Ravussin et al., 1997).

### *Adiponectin*

Adiponectin was discovered about the same time as leptin in the mid-1990s by four research groups independently (Hu, Liang, & Spiegelman, 1996; Maeda et al., 1996; Nakano, Tobe, Choi-Miura, Mazda, & Tomita, 1996; Scherer, Williams, Fogliano, Baldini, & Lodish, 1995). The direct interaction of adiponectin and leptin is not well understood (Tilg & Moschen, 2006). Adipocytes are the main source of adiponectin, but unlike leptin, serum levels of adiponectin decrease with obesity (Fantuzzi, 2005). Adiponectin is thought to play a role in the modulation of glucose and lipid metabolism by affecting tissue insulin sensitivity (Chandran, Phillips, Ciaraldi, & Henry, 2003; Beltowski, 2003; Karbowska & Kochan, 2006).

Average plasma levels of adiponectin range from 5-10  $\mu\text{g}/\text{ml}$  and can range in non-obese subjects from 2-17 $\mu\text{g}/\text{ml}$  with plasma levels lower in men than women (Arita et al., 1999). Plasma levels are negatively correlated with BMI, unlike leptin, which is positively correlated with BMI (Matsuzawa, 2006). Adiponectin exists in different chemical forms in circulation such as multimers, trimers, hexamers or oligomers (Karbowska & Kochan, 2006; Chandran et al., 2003; Tilg & Moschen, 2006). Methods to measure adiponectin include a radioimmunoassay to detect a multimeric form, or an enzyme-linked immunosorbent assay (ELISA) that detects a monomer form. Either method appears to yield similar levels of circulating adiponectin (Chandran et al., 2003).

Serum adiponectin is inversely correlated with insulin resistance (Arita et al., 1999). Low adiponectin levels are more closely related to the degree of insulin resistance and hyperinsulemia than to adiposity (Weyer et al., 2001). Although serum adiponectin and abdominal visceral fat are inversely correlated, there is some thought that this may possibly reflect an underlying link between visceral fat and insulin resistance (Chandran et al., 2003). Adiponectin has been shown to be lower in obese individuals with metabolic syndrome than obese individuals without metabolic syndrome (Xydakis et al, 2004).

Xydakis and coworkers (2004) studied prospectively the association of adiponectin and metabolic abnormalities related to metabolic syndrome. The effects of rapid weight loss on adiponectin, leptin and HOMA-IR were examined. Over a 12 months period, 40 obese individuals with metabolic syndrome (MS+) were compared with 40 obese individuals without metabolic syndrome (MS-), based on three or more ATP III criteria. In total there were 56 women and 47 men,  $47.1 \pm 0.9$  years, with a BMI  $38.3 \pm 0.7$  kg/m<sup>2</sup>. Baseline data was collected after an overnight fast, but without being on a calorie restricted diet. After following a protein sparing, very low calorie diet of 600-800 kcal daily for 4-6 weeks, data collected at baseline was repeated (Xydakis et al., 2004).

Baseline data showed differences in the two groups. HOMA IR values, MS+ to MS-, were  $7.7 \pm 1.5$  to  $3.6 \pm 1.0$  ( $p = 0.0003$ ). Leptin values (pmol/liter), MS+ to MS -, were  $2964 \pm 254$  to  $3111 \pm 232$  ( $p = 0.6$ ). Adiponectin values ( $\mu\text{g/ml}$ ) were  $7.6 \pm 0.6$  to  $10.4 \pm 0.6$  ( $p = 0.003$ ) (Xydakis et al., 2004).

At the 4-6 week data collection, weight loss averaged 7% of initial weight ( $17.6 \pm 1.2$  lbs). In MS+ subjects, weight (lbs) changes, before and after, were  $257 \pm 8.3$  to  $239 \pm 8.0$  ( $p <$

0.0001). HOMA IR changes, before and after, were  $7.7 \pm 1.5$  to  $2.3 \pm 0.5$  ( $p < 0.001$ ). Leptin ( $\mu\text{mol/liter}$ ) changes, before and after, were  $2964 \pm 254$  to  $1727 \pm 219$  ( $p < 0.001$ ). Adiponectin ( $\mu\text{g/ml}$ ) changes, before and after were  $7.5 \pm 0.6$  to  $7.1 \pm 0.5$  ( $p = 0.1$ ). Data for the MS- group was not reported (Xydakis et al., 2004).

Surprisingly, study findings showed that adiponectin levels did not significantly change with a 4-6 week calorie restricted diet in individuals with the metabolic syndrome. However, one important finding noted in this study was that adiponectin levels decreased in parallel to the number of metabolic syndrome components present. Subjects with 4-5 components of the metabolic syndrome had the lowest adiponectin concentrations,  $6.5 \pm 0.9 \mu\text{g/ml}$  (Xydakis et al., 2004). The researchers suggest that adiponectin's inverse association with the expression of metabolic syndrome may somehow reflect adipocyte dysregulation in short term rapid weight loss, rather than a cause of insulin resistance (Xydakis et al., 2004). Chandran and colleagues (2003), in their review of various epidemiological studies, noted an association between insulin resistance and low adiponectin levels, but also acknowledged that it is not yet established whether the dysregulated metabolic state is caused by, or the effect of, low adiponectin levels (Chandran, Phillips, Ciaraldi, & Henry, 2003).

Adiponectin is also recognized to have a wide variety of effects beyond its relationship to insulin sensitization and obesity. It is now thought that adiponectin is also involved in various immune and inflammatory pathologies, functioning as both an anti-inflammatory and pro-inflammatory cytokine (Tilg & Moschen, 2006; Lago et al., 2007).

Adiponectin is induced by up regulation of adiponectin gene expression (Karbowska & Kochan, 2006). The thiazolidinedione (TZD) class of drugs is known to increase adiponectin

expression (Chandran et al., 2003; Karbowska & Kochan, 2006; Matsuzawa, 2006). Peroxisome proliferator-activator receptor gamma (PPAR- $\gamma$ ) is considered a master transcription factor for multiple adipocyte genes (Sharma & Staels, 2007; Chandran et al., 2003; Karbowska & Kochan, 2006). TZD's are specific synthetic ligand activators of PPAR- $\gamma$ , which improve glucose tolerance and insulin sensitivity in type 2 diabetic patients though mechanisms not completely understood (Chandran et al., 2003). PPAR- $\gamma$  may somehow regulate adiponectin synthesis, suggesting that adiponectin may be a biomarker of in vivo PPAR- $\gamma$  activation (Chandran et al., 2003). Adiponectin is noted to increase with administration of the TZD class of medications, such as Rosiglitazone™, used in diabetes.

#### *Interleukin-6*

Interleukin-6 (IL-6) is a cytokine produced in part by adipocytes. It is considered a biomarker of inflammation and is implicated in many pro-inflammatory states (Tilg & Moschen, 2006). High levels of IL-6 may contribute to the increase of acute phase proteins such as C-reactive protein (CRP) seen in obese people (Fantuzzi, 2005). The presence of these acute phase proteins is the reason obesity is considered a chronic inflammatory state (Fantuzzi, 2005; Tilg & Moschen, 2006).

The mechanisms of action of IL-6 are varied and complex. IL-6 modulates insulin activity. As a hormone, IL-6 can act locally or systemically with immune or inflammatory actions (Bulcao et al., 2006). IL-6 can cause a reduction in adiponectin gene expression and secretion (Fasshauer et al., 2003). On the other hand, adiponectin, by its effects on innate and adaptive immunity, can inhibit IL-6 and TNF- $\alpha$  production (Lago et al., 2007). Leptin can be increased by various inflammatory stimuli including IL-6 (Lago et al., 2007).

Increased IL-6 is noted to be present in obese individuals who are insulin resistant and may predict development of type 2 diabetes (Pradham, Manson, Rifai, Buring, & Ridker, 2001; Tilg & Moschen, 2006). In patients who are obese, adipocytes contribute about one third of IL-6 in circulation (Mohamed-Ali et al., 1997). Visceral white adipose tissue appears to produce increased levels of IL-6 compared to subcutaneous fat (Fain, Madan, Hiler, Cheena, & Bahouth, 2004; Fried, Bunkin, & Greenberg, 1998).

In a number of studies, IL-6 is noted to be increased with obesity, and decreased with weight loss. One such study by Bastard and colleagues (2000) investigated the role of adipose cytokines in their relationship to obesity associated insulin resistance in 28 women volunteers [8 lean, no diabetes (BMI, 18.4-23.2 kg/m<sup>2</sup>), 14 obese, no diabetes (BMI, 32.9-48.7 kg/m<sup>2</sup>), and 7 with type 2 diabetes using American Diabetes Association criteria (BMI, 29.0 -39.6 kg/m<sup>2</sup>)]. IL-6 and leptin were measured along with a measurement of fasting insulin slightly different from the HOMA IR. The Fasting insulin resistance index (FIRI) was calculated as (FIRI = Fasting glucose x fasting insulin/25). Blood samples were drawn after an overnight fast (Bastard et al., 2000).

In addition, the 14 obese women without diabetes followed a very low calorie diet program, 941 ± 27 kcal per day, for 21 days. This group had two subcutaneous adipose tissue biopsies taken, before and after the 21 day dietary intervention, using percutaneous mini-liposuction at the level of the umbilicus. Subjects continued the very low calorie diet at the time of the second biopsy. Blood samples collected at baseline were repeated also at this time (Bastard et al., 2000).

Baseline data showed in general that the obese women with and without diabetes were insulin resistant, compared to the 8 lean women without diabetes who were not insulin

resistant. IL-6 and leptin levels were higher in the obese groups with and without diabetes, compared to the lean control group. In the 14 obese women without diabetes BMI ( $\text{kg}/\text{m}^2$ ) improved with the 21 day diet intervention with a mean reduction of  $2.1 \text{ kg}/\text{m}^2$  ( $p < 0.005$ , and also noted was a 3 kg loss of adipose tissue mass ( $p < 0.005$ ). FIRI before and at the end of the 21 day diet, was  $3.04 \pm 0.37$  to  $2.28 \pm 0.26$  ( $p < 0.05$ ). Leptin ( $\text{ng}/\text{mL}$ ), before and at the end of the diet, was  $54.9 \pm 4.5$  to  $29.6 \pm 3.1$  ( $p < 0.005$ ). IL-6 ( $\text{pg}/\text{mL}$ ), before and at the end of the diet, was  $2.78 \pm 0.30$  to  $2.32 \pm 0.19$  ( $p = 0.05$ ). In the subcutaneous tissue biopsies, IL-6 and leptin protein contents decreased significantly with the very low calorie diet respectively by ( $22 \pm 9\%$ ,  $p < 0.05$ , and  $36 \pm 4\%$ ,  $p < 0.01$ ), and reflect those observed at the serum level (Bastard et al., 2000).

This study showed a positive correlation between IL-6 and insulin resistance suggesting a link between IL-6 and obesity and that IL-6 may be involved in insulin resistance. Also, adipose tissue biopsies showed a slight decrease in IL-6 after the very low calorie diet that was associated with slight decreases in serum IL-6 concentrations. Circulating IL-6 levels were associated with obesity, but there was not an association with loss of weight or fat mass and IL-6 changes (data not reported in study). Bastard and colleagues (2000) suggest it is probable that the IL-6 variations reflect the result of the diet intervention, rather than fat mass loss. Decreased leptin was attributed to a reduction in leptin expression and a reduction in production of fat (Bastard et al., 2000).

Bruun and associates (2003) studied the effects of weight loss on anthropometric measures, metabolic parameters and various cytokines. Among items measured were plasma levels of IL-6 and HOMA IR in nineteen obese men (BMI,  $38.6 \pm 0.6 \text{ kg}/\text{m}^2$ ) and ten lean men



(BMI,  $23.4 \pm 0.4 \text{ kg/m}^2$ ). The obese subjects had a weight loss intervention and received a 4.2 MJ/day diet (1003.15 kcal) for 8 weeks, followed by 8 weeks on energy restriction 6.2 MJ/day (1480.84 kcal), and 8 weeks on a weight maintenance diet. Fasting blood work was drawn at baseline for both groups and at the end of the 24 weeks in the obese subject group. Baseline comparison of groups showed obese subjects to be more insulin resistant compared to lean controls ( $p < 0.001$ ). Plasma levels of IL-6 were 64% higher in obese compared to lean controls at baseline ( $p < 0.05$ ). In obese subjects baseline IL-6 was found to be correlated with HOMA IR ( $r = 0.57$ ,  $p < 0.01$ ) and fasting insulin (no r value reported,  $p < 0.05$ ). Change in IL-6 was negatively correlated with change in HOMA IR ( $r = -0.60$ ,  $p < 0.01$ ) (Bruun, Verdich, Toubro, Astrup, & Richelsen, 2003).

After the 24 week intervention, the obese subjects lost 15% of their body weight,  $127.6 \pm 3.2 \text{ kg}$  to  $108.9 \pm 3.8 \text{ kg}$  ( $p < 0.001$ ), and reduced total body fat mass by 30% ( $p < 0.001$ ) and waist circumference by 20% ( $p < 0.001$ ). Decreases in total fat mass and visceral fat mass mirrored insulin sensitivity changes measured by HOMA IR,  $26.0 \pm 2.9$  to  $14.5 \pm 2.1$  ( $p < 0.001$ ). IL-6 decreased by 25%,  $4.1 \pm 0.4 \text{ pg/ml}$  to  $3.1 \pm 0.4 \text{ pg/ml}$  ( $p < 0.001$ ) (Bruun et al., 2003).

In this study, weight loss was associated with a decrease in IL-6. The decrease in IL-6 after weight loss correlated with a decrease in HOMA IR and is in agreement with the decreases in IL-6 and insulin sensitivity observed by Bastard and coworkers (2000) reported above (Bruun et al., 2003).

In a longitudinal intervention study, Gallistl and coworkers (2001) studied changes in IL-6 and leptin concentrations in forty-nine obese children and adolescents during a weight reduction program. Thirty-one obese Caucasian girls (BMI,  $26.9 \pm 5.25 \text{ kg/m}^2$ ) and 18 obese

Caucasian boys (BMI,  $26.2 \pm 5.2$  kg/m<sup>2</sup>) were investigated and compared to sixty-nine lean controls, 28 male and 41 female, (BMI,  $18.5 \pm 3.4$  kg/m<sup>2</sup>). Obesity was defined as a BMI > 85<sup>th</sup> percentile for age and sex. Blood samples were taken at the beginning, and after 3 weeks of the weight reduction intervention program while on calorie restriction. The obese group participated in physical activities for 3 weeks during the summer including physical training 3 times daily. In addition they were assigned to a 3.8 to 5 MJ (908-1194 kcal per day) mixed diet (Gallistl, Sudi, Aigner, & Borkenstein, 2001).

Baseline data comparisons showed control group, IL-6 ( $p < 0.01$ ) and BMI ( $p < 0.0001$ ), significantly lower compared to IL-6 and BMI of obese children. Leptin was not reported. After the intervention, the obese group noted improvements in IL-6, leptin and BMI. Comparing before and after changes, BMI (kg/m<sup>2</sup>) improved from  $26.7 \pm 5.2$  to  $25.3 \pm 5.3$  ( $p < 0.005$ ). The changes in IL-6 (pg/ml) were  $3.9 \pm 4.7$  to  $2.0 \pm 2.2$  ( $p < 0.05$ ). Leptin (ng/ml) changes were  $20.9 \pm 14.9$  to  $5.6 \pm 5.7$  ( $p < 0.005$ ). Changes in leptin were not related to changes in IL-6 concentrations (no  $p$  value reported). Changes in IL-6 correlated with changes in BMI ( $r = 0.25$ ,  $p = 0.03$ ). It was also noted that children and adolescents who had higher baseline IL-6 concentrations had the greatest decreases in IL-6 with the intervention (Gallistl et al., 2001).

This study showed that IL-6 concentrations decreased with restricted energy intake and increased physical activity and improvements of body composition. The findings are in agreement with the findings of Bastard and colleagues (2000) (Gallistl et al., 2001). Gallistl and coworkers, in reviewing their data, noted that the study population was losing weight and not weight stable when serial blood samples were measured. They suggest that further studies in

weight stable individuals be investigated to see if the effects on IL-6 are of longer duration (Gallistl et al., 2001).

### *Interactions between the Cytokines*

IL-6, leptin and adiponectin have interactions that add complexity to understanding their relationship to obesity and insulin resistance. Leptin is known to modulate appetite (La Cava & Matarese, 2004), but also has a pro-inflammatory effect in the immune system, and can be stimulated by IL-6 (Lago et al., 2007). On the other hand, leptin can stimulate production of IL-6 in monocytes and macrophages (Gainsford et al., 1996), which infiltrate adipose tissue in obesity, and amplify the inflammatory state in white adipose tissue (Trayhurn, 2005). As a result, adipocytes release inflammatory cytokines, such as IL-6, which are reflected in elevated serum concentrations in obesity (Trayhurn & Wood, 2004). Adiponectin, on the other hand, can inhibit IL-6 by inhibiting macrophage function (Lago et al., 2007). Conversely, IL-6 has been shown to inhibit adiponectin gene expression (Fasshauer et al., 2003; Brunn, Verdich, Toubro, Astrup, & Richelsen, 2003). The direct interaction between leptin and adiponectin is not clearly understood (Tilg & Moschen, 2006).

### *Correlations of the Cytokines to Obesity and Insulin Resistance*

Some of the correlations of IL-6, leptin and adiponectin to obesity and insulin resistance noted in the literature are summarized below. Serum concentrations of IL-6 are directly correlated with adiposity and insulin resistance (Cottam et al., 2004; Fried, Bunkin, & Greenberg, 1998), and are increased in individuals who are insulin resistant and obese (Tilg & Moschen, 2006). Circulating levels of leptin directly correlate with adipose tissue mass (Friedman & Halaas, 1998; Lago, Dieguez, Gomez-Reino, & Gualillo, 2007). Adiponectin is noted

to have insulin sensitizing properties (Berg, Combs, Du, Brownlee, & Scherer, 2001; Bulcao et al., 2006). Adiponectin circulating levels are reduced in obesity (Arita et al., 1999; Vettor, Milan, Rossato, & Federspil, 2005) and correlate inversely with insulin resistance (Arita et al., 1999; Mojiminiyi, Abdella, Arouji, & Nakhi, 2007).

#### The Effects of Weight Loss and Calorie Restriction on Cytokines and Insulin Resistance

The effect of calorie restriction is hard to separate from weight loss in research studies as weight loss often is the goal of caloric restriction. The effect of calorie restriction is not limited to weight reduction however. Calorie restriction has been shown to extend life span and retard age-related chronic disease in a variety of species, including rats, mice, fish, flies, worms, and yeast (Heilbronn & Ravussin, 2003). The majority of research done related to calorie restriction in humans or animal studies has focused on the effect of calorie restriction on aging.

In an exhaustive review of over 1500 papers since the 1960's, Barzilai and Gupta (1999) identified many of the positive effects of food restriction on life extension and in various systems or organs in aging animals. A further review, conducted in 2003 by Heilbronn and Ravussin, identified many effects of chronic caloric restriction on aging including decreased circulating insulin and body temperature, decreased total energy expenditure, decreased oxidative stress, fat mass, and free fat mass, as well as visceral fat. Also noted in the literature review were decreases in intramyocellular lipids and increased insulin sensitivity (Heilbronn & Ravussin, 2003). There are, however, limited studies in the literature that include a weight maintenance period following weight loss after a calorie restricted diet intervention.

#### *Weight Loss without Calorie Restriction*

The recent increase in gastric bypass surgery and liposuction has allowed researchers to

study the effects of surgery on weight loss, insulin resistance and various cytokines.

### *Liposuction*

Liposuction has been proposed as a treatment for the metabolic complications of obesity and was the subject of a study by Klein and colleagues (2004). The purpose was to determine the effect of large volume liposuction on insulin sensitivity in liver, muscle and adipose tissue, as well as look at risk factors for coronary heart disease in serum measures of insulin, leptin, adiponectin, and IL-6 (Klein et al., 2004).

Fifteen obese women with abdominal obesity underwent large-volume liposuction, which is defined as removal of > 4 liters of aspirate (Trott et al., 1998). Eight of the women had normal glucose tolerance with a mean BMI of  $35.1 \text{ kg/m}^2 \pm 2.4$  and seven had type 2 diabetes and a mean BMI of  $39.9 \text{ kg/m}^2 \pm 5.6$  (Klein et al., 2004). Subjects with normal glucose tolerance had a total of 16.1 liters of aspirate ( $12 \pm 1$  liters from the upper body and  $4 \pm 2$  liters from the lower body) removed, and subjects with type 2 diabetes had a total of  $17 \pm 2$  liters ( $16 \pm 2$  liters from the upper body and  $1 \pm 1$  liters from the lower body) (Klein et al., 2004).

Subjects were evaluated before, and 10-12 weeks after liposuction, and were weight stable before and after liposuction. The researchers measured insulin sensitivity by a euglycemic-hyperinsulinemic clamp procedure which included isotope-tracer infusions (Klein et al., 2004). Somewhat different from the euglycemic clamp procedure described earlier, this clamp procedure was divided into two stages with varied rates of infusion of insulin, and isotope tracers. The protocol offers the advantage of evaluating the effect of insulin on glucose production (liver), and lipolysis (adipose tissue) (stage 1), and on glucose disposal (skeletal muscle) (stage 2) (Klein et al., 2004).

Serum measures of plasma insulin and leptin were measured by radioimmunoassay, and IL-6 and adiponectin were measured by enzyme-linked immunoabsorbent assay [ELISA] (Klein et al., 2004). Liposuction did not have a significant effect on the insulin sensitivity of muscle, liver, or adipose tissue (no p value reported), nor have significant effects on serum concentrations of IL-6 or adiponectin. The mean changes before and after liposuction for IL-6 in the normal glucose group was +0.9 pg/ml ( $p = 0.10$ ), and the diabetes group -0.7 pg/ml ( $p = 0.24$ ). For adiponectin, mean changes for the normal glucose group was -0.5 ng/ml ( $p = 0.13$ ), and for the diabetes group -0.7 ng/ml ( $p = 0.13$ ). Leptin serum concentration levels were decreased in the normal glucose tolerance group -8.2 ng/ml ( $p = 0.05$ ), and in the type 2 diabetes group -5.5 ng/ml ( $p = 0.05$ ) (Klein et al., 2004).

Liposuction decreased the volume of subcutaneous abdominal adipose tissue by 44 percent in the normal glucose tolerance group, but only 28 percent in the diabetes group. Volumes of visceral adipose tissue and thigh adipose tissue did not change significantly. In the 10-12 week after surgery measurements, body fat mass decreased  $9.1 \pm 3.7$  kg ( $18 \pm 3$  % of total fat mass,  $p = 0.002$ ) from baseline in the normal glucose tolerance group, and decreased  $10.5 \pm 3.3$  kg ( $19 \pm 2$  % of total fat mass,  $p < 0.001$ ) in the type 2 diabetes group. The decrease in fat mass was consistent with fat mass aspirated during liposuction (which is considered to be 60% of the total liposuction aspirate) (Klein et al., 2004).

A total weight loss of 12% (by conventional means) equals the weight loss achieved in this study by liposuction (Klein et al., 2004). Removing large amounts of abdominal subcutaneous fat (but not visceral fat) did not improve insulin sensitivity, nor significantly

change plasma concentrations of IL-6 and adiponectin. The decrease in leptin was thought to reflect changes in adipose tissue mass (Klein et al., 2004).

By contrast, a prospective study from Europe investigated the role of liposuction on circulating inflammatory markers in 30 non-diabetic obese premenopausal females, mean BMI  $34 \text{ kg/m}^2 \pm 2.7$ . Baseline data, including insulin sensitivity by HOMA, IL-6, and adiponectin, were collected in the obese group before liposuction, and compared to a control group of 30 age-matched normal weight women, mean BMI  $23.7 \text{ kg/m}^2 \pm 1.2$ . Six months after liposuction, the obese group had baseline data repeated. The obese group was encouraged to follow a weight maintenance diet following liposuction (Giugliano et al., 2004).

The mean aspirate volume of liposuction was 3540 ml (range 2550-4670 ml) corresponding to a net lipid loss of  $2.7 \pm 0.7 \text{ kg}$  (mean  $\pm$  SD). Mean weight change was  $-3 \text{ kg}$  ( $p < 0.001$ ) (Giugliano et al., 2004). After 6 months, in a weight stable state, the obese women were less insulin resistant (mean change HOMA  $-1.02$ ,  $p < 0.05$ ), had decreased insulin (mean change  $-4 \mu\text{U/ml}$ ,  $p < 0.05$ ), had reduced IL-6 (mean change  $-0.9 \text{ pg/ml}$ ,  $p < 0.05$ ), and increased adiponectin (mean change  $+1.3 \mu\text{g/ml}$ ,  $p < 0.02$ ). There was a significant correlation between amount of fat aspirate and HOMA ( $r = 0.28$ ,  $p < 0.05$ ), and the amount of fat aspirate and adiponectin ( $r = -0.34$ ,  $p < 0.02$ ) (Giugliano et al., 2004).

In contrast to the study by Klein, et al. (2004), Giugliano and colleagues (2004) noted increased changes in IL-6, adiponectin, and insulin sensitivity following liposuction. The mean volume of aspirate during liposuction is much less in comparison to the study by Klein, et al. (2004). Leptin was not measured. The study by Klein, et al. (2004) involved 15 obese women, 7 of which had type 2 diabetes. The study by Giugliano, et al. (2004) had 30 obese women that

underwent liposuction, with follow up at 6 months. Participants were encouraged to follow a weight maintenance diet at 6 months. Klein and colleagues (2004) follow up measures were at 3 months while weight stable. The amount of aspirate differed between the two studies, which might contribute to the conflicting results.

### *Gastric Bypass Surgery*

Vendrell, et al. (2004) studied the effect of bypass surgery on interactions between various cytokines in 57 morbidly obese men and women (mean BMI  $49.3 \text{ kg/m}^2 \pm 7.4$ ) who underwent gastric bypass surgery. Baseline data was collected and compared to 117 obese subjects (mean BMI  $32.9 \text{ kg/m}^2 \pm 7.4$ ). Data collected at baseline was repeated 24 weeks after surgery in 34 of the 57 morbidly obese group subjects who agreed to complete a follow-up examination. Adiponectin, leptin and IL-6, fasting plasma insulin and HOMA-IR were part of the data collected at baseline and follow-up. The study does not indicate if subjects were weight stable or continuing to have weight loss at the 24 week follow up (Vendrell et al., 2004).

Comparing baseline data from the obese group to the morbid group respectively: fasting insulin was  $15.2 \text{ } \mu\text{U/ml} \pm 7.9$  and  $20.4 \text{ } \mu\text{U/ml} \pm 9.0$  ( $p < 0.05$ ); HOMA-IR  $3.4 \pm 2.1$  and  $5.7 \pm 3.9$  ( $p < 0.05$ ); median data (75<sup>th</sup> percentile) for IL-6  $1.3 \text{ pg/ml}$  (1.7) and  $7.6 \text{ pg/ml}$  (19.4) ( $p < 0.05$ ); adiponectin  $16.2$  (19.6) and  $19.6$  (36.6) ( $p < 0.05$ ) (Vendrell et al., 2004).

Weight loss ranged from 20-39.7% after surgery. Mean weight changes  $129.8 \text{ kg} \pm 24.4$  pre and  $91.3 \text{ kg} \pm 16.2$  post surgery ( $p < 0.001$ ). BMI changes were  $49.6 \text{ kg/m}^2 \pm 5.9$  pre to  $34.9 \text{ kg/m}^2 \pm 4.1$  ( $p < 0.001$ ). Insulin and HOMA-IR decreased significantly ( $p < 0.001$ ) with weight loss in the 34 subjects included in the follow-up at 24 weeks. Changes in fasting insulin were  $20.3 \text{ } \mu\text{U/ml} \pm 11.2$  pre, to  $7.3 \text{ } \mu\text{U/ml} \pm 3.7$  post, at 24 weeks. HOMA-IR changes were  $5.5 \pm 3.5$



pre, to  $1.5 \pm 0.9$  post, at 24 weeks. HOMA-IR values were associated with the increase in adiponectin ( $p = 0.014$ ) after weight loss (Vendrell et al., 2004).

Adiponectin increased significantly ( $p < 0.05$ ), and leptin and IL-6 decreased significantly ( $p < 0.001$ ) after surgery. Median (75<sup>th</sup> percentile) changes in adiponectin were  $20.7 \mu\text{g/mL} \pm 39.4$  pre, to  $40.9 \mu\text{g/mL} \pm 53.6$  post; IL-6 changes were  $5.1 \text{ pg/mL} \pm 18.3$  pre, to  $2.1 \text{ pg/mL} \pm 7.2$  post; and leptin changes were (mean  $\pm$  SD)  $59.6 \mu\text{g/L} \pm 26.2$  pre to  $18.8 \mu\text{g/L} \pm 9.7$  post intervention (Vendrell et al., 2004).

#### *Bariatric Surgery Treated Obese Subjects and Conventional Weight Loss Treated Obese Subjects*

A prospective, non-randomized intervention trial from Sweden, compared obese subjects who underwent bariatric surgery to conventionally treated (non surgical) obese control subjects, to see if short term benefits of weight loss persist over time. Overall, 1703 subjects were followed for two years and 4047 subjects were followed for 10 years. The mean BMI was  $41 \text{ kg/m}^2$  for all participants. Participants were matched in a sequential treatment assignment to a surgical group or a conventional non surgical treatment control group. Subjects who completed the 10 year study included control group ( $n=627$ ) and surgical group ( $n=641$ ). Subjects who completed the 2 year study included control group ( $n=1660$ ) and surgical group ( $n=1845$ ) (Sjostrom et al., 2004).

The surgical group underwent one of three bariatric procedures: gastric bypass, vertical banded gastroplasty, or banding. The controls received the nonsurgical treatment for obesity that was customary for the center at which they were registered, with no attempt at standardization. Interventions varied from center to center (Sjostrom et al., 2004). Weight after two years, compared to baseline, increased 0.1% in the control group, and decreased 23.4% in

the surgical group ( $p < 0.001$ , difference between groups at 2 years). After 10 years, weight had increased from baseline 1.6% in the control group, and decreased in the surgical group 16.1% ( $p < 0.001$ , difference between groups at 10 years) (Sjostrom et al. 2004).

Glucose and insulin levels were increased in the control group, but decreased in the surgical intervention groups at both 2 and 10 years. Insulin changes at two years compared to baseline in the controls was +10.3 %; and the surgical group change from baseline at two years was -46.2 %; a between group difference of 51.4% ( $p < 0.001$ ) (Sjostrom et al. 2004).

At ten years the change from baseline in controls was +12.3%; the surgical group change at 10 years from baseline was -28.2%, a difference of 30.3% ( $p < 0.001$ ) between groups at 10 years (Sjostrom et al., 2004).

The surgical group had increased rates of recovery from diabetes as well as lower incidence rates of diabetes, compared to the conventionally treated controls over a 10 year period, in addition to greater weight loss, increased physical activity, and lower energy intake (Sjostrom et al., 2004).

### *Weight Loss with Calorie Restriction*

An exhaustive literature review on the long term effects of calorie restriction by Heilbronn and Ravussin (2003) and the earlier review by Barzilai and Gupte (1999) led to a number of randomized control trials (RCT), part of a larger CALERIE-Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy study.

#### *6 Month Calorie Restriction and Weight Loss in Sedentary, Non Obese Men and Women*

Heilbronn and colleagues (2006) examined the effects of 6 months of calorie restriction,

with or without exercise in healthy, sedentary, men and women, who were overweight but not obese (BMI, 25 to < 30 kg/m<sup>2</sup>) (Heilbronn et al., 2006). In this RCT, 48 participants were randomly assigned to one of four groups for 6 months: control or weight maintenance group; a 25% calorie restriction from baseline energy requirement group; a 12.5% calorie restriction and 12.5% increase in energy expenditure group; and a very low-calorie diet group (890 kcal/d until 15 % weight reduction followed by a weight maintenance diet). In the very low calorie diet group, the target 15% weight reduction was achieved by week 8 in men and week 11 in women (Heilbronn et al., 2006).

Weight loss at 6 months compared to baseline was: controls, -1.0% (1.1%); calorie restriction group, -10.4% (0.9%); calorie restriction with exercise group -10.8% (0.08%); and very low-calorie diet group, -13.9% (0.7%). Fasting insulin levels were significantly reduced from baseline at 3 months in the calorie restriction, and the calorie restriction with exercise groups only ( $p < 0.01$ ), but not in the very low calorie group, which was weight stable at 3 months. Fasting insulin levels were significantly reduced in all the intervention groups ( $p < 0.01$ ) at 6 months compared to baseline (Heilbronn et al., 2006).

#### *12 Month Calorie Restriction and Weight Loss in Sedentary, Non Obese Men and Women*

Another 12 month RCT, also associated with the CALERIE investigation, studied whether exercise training-induced weight loss improved glucose tolerance and insulin action, to a greater extent, than similar weight loss through calorie restriction. Adiponectin was also measured (Weiss et al., 2006). The investigators randomly divided 46 sedentary, but not obese, men and women (BMI 23.5-29.9 kg/m<sup>2</sup>), age 50-62 years, into 3 groups: a 12 month exercise training group (EX); a 12 month calorie restriction group (CR); and a healthy lifestyle control

group (HL). Individuals were excluded if they had a history of diabetes or fasting blood glucose at baseline of  $\geq 126$  mg/dL (Weiss et al., 2006).

The CR group decreased caloric intake by 16% compared to baseline during the first 3 months, and 20% during the remaining 9 months. The EX group kept energy intake constant at baseline levels, and increased energy expenditure by 16% for the first 3 months, and 20% for the remaining 9 months. The HL group did not receive instructions to change diet or exercise behaviors (Weiss et al., 2006).

At the end of one year, total body weight decreased in the EX group by  $6.6 \text{ kg} \pm 5.5$  ( $p \leq 0.05$ ); in the CX group by  $8.2 \text{ kg} \pm 4.8$  ( $p \leq 0.05$ ); and in the HL group by  $1.2 \text{ kg} \pm 2.1$  (no p value reported) (Weiss et al., 2006). Both the EX and CR groups showed improvements in fasting insulin: the EX group  $-2.7 \text{ } \mu\text{U}/\text{mL} \pm 5.0$  ( $p \leq 0.05$ ), and the CR group  $-2.5 \text{ } \mu\text{U}/\text{mL} \pm 3.9$  ( $p \leq 0.05$ ). The HL group showed no improvement:  $1.3 \text{ } \mu\text{U}/\text{mL} \pm 3.2$  (no p value reported) (Weiss et al., 2006).

Insulin sensitivity, measured by an insulin sensitivity index (ISI), increased in both the EX  $3.0 \pm 2.7$  ( $p \leq 0.05$ ), and CR  $2.0 \pm 3.9$  ( $p \leq 0.05$ ) groups, but not in the HL  $0.3 \pm 1.4$  (no p value reported) group at the end of the 12 month intervention (Weiss et al., 2006).

Weiss et al (2006) also measured adiponectin levels, and found fasting serum concentrations increased in the EX group ( $p = 0.06$ )  $1.9 \text{ } \mu\text{g}/\text{mL} \pm 4.0$ , and the CR group ( $p = 0.07$ )  $2.2 \text{ } \mu\text{g}/\text{mL} \pm 4.7$ , but decreased in the HL group ( $p = 0.05$ )  $1.9 \text{ } \mu\text{g}/\text{mL} \pm 2.6$  (Weiss et al., 2006). Adiponectin levels were higher in the EX and CR groups than the HL group at the end of the 12 month intervention, but not significantly different between the EX and CR groups (no p value reported).

Changes in ISI in the EX and CR were correlated to changes in adiponectin levels. No significant difference ( $p = 0.37$ ) was noted between the EX group ( $r = 0.47$ ) and CR group ( $r = 0.17$ ) with respect to correlations between change in adiponectin and change in ISI (Weiss et al., 2006).

In summary, Weiss and colleagues (2006) showed that weight loss by 12 month exercise training or by 12 months of calorie restriction resulted in improvements in insulin action that was not significantly different between the two groups. Each intervention resulted in improvements in circulating adiponectin compared to the healthy lifestyle intervention. Participants were overweight but not obese (Weiss et al., 2006).

#### *Weight Loss in Obese Women*

In comparison, Ross and colleagues (2004) conducted a RTC of 54 premenopausal women with abdominal obesity (mean BMI averaged  $31.3 \pm 2.0$  kg/m<sup>2</sup>). They studied and compared to a weight stable control group, the effects of equivalent diet induced weight loss, exercised induced weight loss, and exercise without weight loss, on subcutaneous fat, visceral fat, and insulin sensitivity (Ross et al., 2004).

Subjects, after being randomly assigned, followed a weight maintenance diet for a 4-5 week baseline period before beginning the 14 week intervention. The control group was asked to maintain weight during the 14 weeks. The diet weight loss group reduced caloric intake by 500 kcal/day for the treatment period. The exercise weight loss group maintained the same level of diet as the baseline period during the 14 week intervention, but combined exercise that expended 500 kcal/day. The protocol was designed to achieve a weight loss goal of 1.0 lb (0.45 kg) per week in the exercise group. Subjects in the exercise without weight loss group were

asked to maintain body weight and consumed calories to compensate for the energy burned during exercise (~500 kcal) (Ross et al., 2004).

Exercise sessions were monitored and supervised for both exercise groups. All subjects were free living, self selected foods, and kept detailed daily food records. At the end of the intervention, subjects followed a one week weight stabilization period. Insulin sensitivity was measured by euglycemic clamp while the subjects were on a weight maintenance diet for a minimum of 4 days. Subjects avoided strenuous activity for 3 days preceding the baseline studies, and before studies done in the weight maintenance period post intervention. Subjects were hospitalized the night before the studies were performed. A 2 hour OGTT was administered the morning after an overnight fast pre intervention, and ~6 days post intervention in a weight maintenance state (Ross et al., 2004).

Weight changes pre to post treatment (difference between reported means) were: control group -0.5 kg (% changes not reported in all groups); diet weight loss group -6.5 kg; exercise weight loss -5.9 kg; exercise without weight loss -0.5 kg. The control group and exercise without weight loss group had no change in body weight. There was no difference ( $p > 0.05$ ) in the average weight loss in the diet weight loss and exercise weight loss groups, which represented a loss of ~6.5% of initial body weight (Ross et al., 2004).

Fasting insulin changes pre to post treatment (differences between reported means) were: control group -5.5 pmol/L (% changes not reported for all groups); diet weight loss -7.0 pmol/L; exercise weight loss -27.9 pmol/L; exercise without weight loss -5.8 pmol/L. Pre and post treatment insulin values during the last 30 minutes of clamp studies were not different within or among groups ( $p > 0.05$ , data not shown in study). The change in OGTT-insulin area

within the exercise weight loss group was greater ( $p < 0.008$ ) in comparison with the control group, but did not change within the diet weight loss and exercise without weight loss groups ( $p > 0.1$ ). Insulin sensitivity, compared to controls, improved within the exercise weight loss group alone ( $p < 0.001$ ). The exercise weight loss group experienced a 32% reduction in insulin resistance. The diet weight loss group did not experience an improvement in insulin sensitivity (Ross et al., 2004).

### *Weight Loss in Obese Men*

Ross and colleagues (2000) also conducted a RCT with obese men, that mirrors the study design of the RTC discussed above involving obese women. Obese men ( $n = 52$ ) with mean BMI of  $31.3 \text{ kg/m}^2$  were randomly assigned to one of four groups: control; diet weight loss; exercise weight loss; and exercise without weight loss, and observed for 3 months. Participants followed a weight maintenance diet before the 12 week intervention, and for two weeks after the intervention to stabilize weight. In contrast to previous study by Ross and colleagues (2004), the diet weight loss group reduced caloric intake by 700 kcal/d from the 4-5 week baseline. The exercise weight loss group maintained baseline diet calorie intake, but increased energy expenditure to 700 kcal/d. Participants in the control group were told to maintain weight. The exercise without weight loss group maintained the baseline diet and consumed the calories expended in exercise (approximately 700 kcal/d). Euglycemic clamp was used pre and post intervention, while participants were in a weight maintenance state. OGTT was also done pre and post intervention in weight maintenance, with the post intervention measurements done ~ 6 days after the intervention (Ross et al., 2000).

Weight loss in each group, pre and post treatment (difference between reported

means), was: control group +0.10 kg; diet weight loss group -7.4 kg; exercise weight loss group -7.5 kg; exercise without weight loss group -0.5 kg. The average weekly weight loss was 0.06 kg for the diet weight loss group and the exercise weight loss group. Total average weight loss for the diet weight loss group was 7.4 kg, and for the exercise weight loss group 7.6 kg, which respectively is ~ 8% of body weight. In this study, an 8% diet or exercise induced weight loss was associated with similar reductions in abdominal obesity, visceral fat, and waist circumference (Ross et al., 2000).

The differences, pre and post treatment (differences of reported means), of fasting insulin were: control group +2.0 pmol/L (% changes not reported in each group); diet weight loss group -10.0 pmol/L; exercise weight loss group -29.0 pmol/L; exercise without weight loss group -10.0 pmol/L. Plasma insulin values, pre and post treatment, in the last 30 minutes of clamp studies did not differ within groups ( $p > 0.05$ , data not shown in study) (Ross et al., 2000). Insulin area values were greater in the exercise weight loss group compared to the exercise without weight loss group ( $p = 0.01$ ). In this study, when diet weight loss is carefully matched to exercise weight loss, reductions in insulin resistance are similar (Ross et al., 2000).

#### *Weight Maintenance Following Calorie Restriction and Weight Loss*

There is limited research in humans measuring markers in a weight maintenance state following caloric restriction. One such study from Europe measured glucagon-like peptide 1 (GLP-1) release before, after weight loss, and after a 3 month weight maintenance phase. Adam and colleagues (2006), in a repeated measures design study, investigated GLP-1 release in 32 obese subjects on three occasions: before weight loss (T1), after a 6 week very low energy diet of 2540 kJ/day (607 kcal)(T2), and after a 3-month weight-maintenance period (T3).



Plasma insulin and HOMA IR were also measured. Blood samples were drawn after an overnight fast at each time period. There was no control group for comparison (Adam, Lejeune, & Westerterp-Plantenga, 2006).

Mean body weight changes, comparing the three time periods, were T1 (86.7 kg, SD 8.8), T2 (79.7 kg, SD 8.4), and T3 (80.7 kg, SD 9.6) ( $p = 0.001$ ). Changes in BMI were T1 (30.0 kg/m<sup>2</sup>, SD 2.5), T2 (27.6 kg/m<sup>2</sup>, SD 2.3), T3 (27.9 kg/m<sup>2</sup>, SD 2-3) ( $p = 0.0001$ ). There were no differences observed in fasting insulin values (measurement units not given) between T1 (11.5  $\pm$  1.08), T2 (11.5  $\pm$  0.8), and T3 (11.6  $\pm$  1.2) (no  $p$  values reported). HOMA IR showed no differences comparing T1 (2.54  $\pm$  0.25) and T2 (2.53  $\pm$  0.26). T3 was not reported. No  $p$  values were reported. The researchers found an unexpected rebound of GLP-1 after the weight maintenance period, but a transient decrease after weight loss which they attributed to a negative energy balance (Adam et al., 2006).

This study showed a return to baseline of GLP-1 despite weight loss that was essentially maintained. Fasting insulin and HOMA IR showed no changes over the three time intervals. There is little literature that studies the long term effects of weight loss maintenance on insulin and various cytokines.

Another study by Weinsier and coworkers (2000), investigated energy expenditure in 32 black and white women, comparing measures done after 4 weeks in an overweight state, BMI 27-30 kg/m<sup>2</sup>, and after 4 weeks in a normal weight state, after loss of  $\geq 10$  kg, and a BMI of  $< 25$  kg/m<sup>2</sup>. The study found decreases in sleeping and resting energy expenditure (EE) in weight-reduced (but weight-stable) black and white women following a weight loss diet, proportional to changes in body composition, and not likely due to disproportionately low

sleeping or resting energy requirements in previously overweight persons (Weinsier et al., 2000).

The significance of this study is that measurements of energy expenditure were made after a 4 week period in both an overweight state and after a 4 week period in weight reduced state. At each time measurement the weight was maintained and stable for the 4 week period.

#### Weaknesses of the Literature

The increasing problem of obesity and its deleterious effect on health and healthcare costs is generally recognized. The underlying mechanisms relating obesity to insulin resistance and other risk factors associated with the metabolic syndrome are unknown and the continued subject of much study. In addition, there is disagreement among expert panels regarding criteria defining the metabolic syndrome.

Obesity is defined using an index of weight adjusted for height expressed in  $\text{kg}/\text{m}^2$ . Yet the BMI is limited in that it gives us no information about the distribution of body fat, such as central adiposity, which is more related to insulin resistance than peripheral adiposity. Waist to hip ratios and waist circumference measures allow some distinction of central versus peripheral adiposity, or an “apple” versus “pear” weight distribution, yet the measures are unable to separate central adiposity into subcutaneous and visceral components. That is best done using computed tomography (CT) or magnetic resonance imaging (MRI) which are costly and not readily available to the researcher or clinician. Hence, there are variances in the research articles as to how fat mass is measured and determined.

Weight loss in turn may be reported as loss in weight, or changes in BMI. Others may include waist circumference or waist to hip ratio as additional measurements, and still others

may use some measure of imaging to assess weight or fat loss.

Insulin resistance can be measured by various means, such as the euglycemic clamp, considered the gold standard, though such measurement is limited to research facilities and not readily available to all researchers. Hence, measurements of insulin resistance vary depending on the research method used.

Given the role of adipose tissue as an endocrine organ, much research has looked at the many different cytokines produced by adipose tissue. Often a cause and effect relationship is sought to help establish the associations between obesity, insulin resistance, weight loss and the multiple activities and roles the various cytokines play. Though many associations and some correlations have been established, underlying mechanisms remain unknown.

The systems theory model hypothesizes that HOMA IR, leptin and IL-6 will decrease with weight loss, while adiponectin would increase with weight loss, though not all studies have consistent results. The proposed model attempts to understand the relationships of the cytokines studied in the context of a calorie restricted diet intervention in people who are overweight and obese and at risk for type 2 diabetes. It is not possible to depict every interaction among the cytokines and their possible changes in relationship to obesity, insulin resistance and weight loss.

Many of the studies involving calorie restriction or weight loss interventions, measure the effect of their interventions in a weight loss or calorie restricted state. Some conduct measurements during a weight maintenance period following the weight loss or calorie restricted intervention, but the periods of time differ from days to weeks to perhaps months. There is little known about the effect of, or ideal length of time for a post intervention follow

up, to accurately assess changes in, for instance, plasma levels of insulin or cytokines, that are representative, and reflect changes long term.

### Research Questions

Despite limited research looking at weight maintenance following a low calorie dietary intervention, there are some studies that measure serum concentrations of HOMA IR, leptin, IL-6 and adiponectin in a weight maintenance period following weight loss. Cytokine dysregulation has been implicated with obesity and insulin resistance and its related pathologies. Further research is needed to understand the long term effect of weight maintenance on insulin resistance, and cytokine regulation. The research questions for this study are as follows:

1. Is there a significant correlation between weight, HOMA IR, IL-6, adiponectin and leptin at baseline?
2. Is there a difference between weight, serum measurements of HOMA IR, IL-6, leptin and adiponectin collected at baseline, after a 28 day 50% caloric restricted diet, and at the end of a 6-8 month post diet period?
3. Is there a relationship between change scores in weight, HOMA IR, IL-6, leptin and adiponectin at baseline and after caloric restriction?
4. Is there a relationship between change scores in weight, HOMA IR, IL-6, leptin and adiponectin after calorie restriction and at the end of the 6-8 month post diet period?

### Definition of Terms

*Metabolic Syndrome* is an association of risk factors including obesity, insulin resistance, dyslipidemia, and high blood pressure first described by Reaven, Lithel and Landsberg, (1996).

*Insulin Resistance*: an impaired biological state in which cells or tissue become resistant to the effects of increasing levels of insulin to maintain normal plasma glucose homeostasis.

*Type 2 Diabetes*: a medical disorder that is the end result of insulin resistance in which glucose uptake into cells is impaired, along with decreased insulin production by pancreatic  $\beta$  cells, resulting in elevated plasma glucose levels or hyperglycemia.

*Homeostasis Model Assessment of Insulin Resistance (HOMA IR)*: A method of measuring insulin resistance. The HOMA IR formula is the product of fasting plasma insulin (FIRI)  $\mu\text{U/ml}$  x fasting plasma glucose (FPG)  $\text{mmol/l}$  divided by 22.5 as defined by Mathews (Mathews et al., 1985).

*Euglycemic Clamp*: a measure of insulin sensitivity to exogenous insulin involving a primary infusion of insulin to raise plasma concentrations to 100  $\mu\text{U/mL}$  while plasma glucose is held constant by a variable infusion. The result is a steady state of euglycemia in which the glucose infusion rate equals glucose uptake by all the tissues of the body (DeFronzo, Tobin, & Andres, 1979).

*Cytokine*: the term used in this study to refer to IL-6, adiponectin and leptin. The term *cytokine* or *chemokine* is used to refer to substances produced in part by adipose tissue (Fantuzzi, 2005) such as interleukin-6 (IL-6), whereas the term *adipokine* or *adipocytokine* is a term used to designate substances primarily produced by adipocytes (Fantuzzi, 2005) such as adiponectin and leptin. Serum levels of leptin, IL-6 and adiponectin were measured in this study using Quantikine Immunoassay ELISA analysis.

#### Significance to Nursing

Obesity and its increasing health burden and costs are known. Obesity is associated

with risk factors related to the metabolic syndrome and diabetes. Nurses are involved in educating and managing patients with obesity, diabetes, metabolic syndrome and other diseases and conditions related to obesity. As providers and advocates, nurses can positively educate and counsel patients who are obese, at risk for being obese, or enable life style changes, weight loss and prevention strategies. To effectively counsel and assist patients, one must understand the pathophysiology related to obesity.

The major problem many people face with weight loss is that they are unable to maintain the weight loss long term. It is now known that cycles of weight loss and gain may actually be detrimental and contribute to long term health risks. Regaining of weight after weight loss may result in deposition of fat that is more metabolically active and centrally located (Rodin, Radke-Sharpe, Rebuffe-Scrive, & Greenwood, 1990). Nurses need to not only understand the complex regulation of adiposity or adipose tissue, but also understand the long term effects of weight maintenance to guide and educate patients in weight loss and dietary interventions.

Such a realization was echoed by Thomas Wadden et al. in 1999. "Diet and exercise are the most common interventions for obesity. If physicians are to advise patients...then studies are needed that demonstrate such losses are associated with long term improvements....Is this relationship the same when patients are in the process of losing weight, regaining it, or are weight stable?" (Wadden, Anderson, & Foster, 1999, p. 171).

## CHAPTER TWO

### METHODS OF STUDY

#### Introduction

This study was part of a larger pilot study investigating the effect of a weight loss diet on risk factors for developing diabetes. The current study examined the effect of caloric restriction on serum levels of the cytokines IL-6, leptin, and adiponectin, weight and HOMA IR, before a weight loss diet intervention (T1), and at the end of the weight loss intervention (T2), and in a 6 to 8 month post diet period (T3), in a subset of the larger convenience sample of 20 overweight and obese men and women ( $BMI \geq 26 \text{ kg/m}^2$ ), aged 18 to 65, who were at risk for type 2 diabetes based on family history.

#### Study Design

A prospective, repeated-measures design was used in this study. Differences between weight, HOMA IR, leptin, adiponectin, and IL-6 were investigated in 9 obese adults, with a family history of diabetes, who provided samples for the three time comparisons.

#### Setting

Two settings in Spokane, Washington were used for this study. Initial interviews, data collection, and assessments took place at the WSU-Spokane Health Sciences Building (WSU-HSB) research facility. Assessments and interviews were conducted under privacy to ensure accurate and reliable data. Blood samples were collected by trained phlebotomists and processed at the same Pathology Associates Medical Laboratory, located at Fifth and Brown.

#### Population and Sample

Using a convenience, nonprobability-sampling method, this study recruited overweight

and obese individuals with a family history of diabetes. A total of twenty subjects were recruited for the study using advertisements placed in a local endocrinology practice, a local hospital, a health maintenance organization clinic, as well as the Intercollegiate College of Nursing. There were additional contacts identified through contact with those enrolled. Enrollment was on a volunteer basis. Any individual who had a first-degree relative with type 2 diabetes, a BMI of at least 26 kg/m<sup>2</sup>, and between the ages of 18-65 was invited to participate in the study. A first degree relative was defined as a person's parents, sibling or child. Informed consent was obtained prior to the collection of any data. A total of twenty women and men were enrolled to provide an adequate amount of data to study differences and correlations between variables over the pilot study time period.

Exclusion criteria included pregnancy, a history of diabetes, fasting blood glucose  $\geq$  126 mg/dl, and current use of medications that would affect measured parameters including steroids and hypoglycemic agents. Individuals were also excluded if they smoked or took immunosuppressive or thyroid medications.

#### Measurement and Instrumentation

##### *BMI*

The participants body weight was measured on the same digital scale at the research office located in the Health Sciences Building. Body weight was measured to the nearest 0.1 pound with the participants in street clothing and stocking feet. The scale was zeroed prior to measurement. BMI was calculated using the following formula: BMI kg/m<sup>2</sup>= [weight (pounds)/height (inches)<sup>2</sup> ] x 703 (Centers for Disease Control and Prevention, 2007).

Federal guidelines use BMI to classify an individual as being *overweight* or *obese*.



*Overweight* is a BMI from 25.0-29.9 kg/m<sup>2</sup> and *obese* is a BMI  $\geq$  30 kg/m<sup>2</sup> (Centers for Disease Control and Prevention, 2007). BMI has been shown to correlate to direct measures of body fat, though it is not a direct measure (Prentice & Jebb, 1985). It is one of the best and most commonly used screening tools to assess overweight and obesity (Centers for Disease Control and Prevention, 2007).

#### *Serum concentrations of cytokines*

Blood samples, following 12 hour fasts by participants, were collected in the morning between 7:30 a.m. and 9:00 a.m. by trained Pathology Associates Medical Laboratory (PAML) phlebotomists using standard technique. The PAML laboratory performed measures of fasting insulin and glucose. The laboratory phlebotomists collected and processed serum for quantification of adiponectin, leptin, and IL-6 in the university research laboratory. Samples were collected in serum separator tubes, allowed to clot for 30 minutes, and then centrifuged 15 minutes at 1000 x g. The serum supernatant was then parceled into cryovials and then frozen at -80° F until assays were performed.

#### *Insulin resistance*

Insulin resistance (IR) was calculated using the homeostatic model (HOMA). The formula for HOMA IR is the product of fasting plasma insulin (FIRI)  $\mu$ U/ml x fasting plasma glucose (FPG) mmol/l divided by 22.5 (Mathews et al., 1985).

#### *Quantikine Immunoassays*

Commercially available Quantikine<sup>®</sup> Immunoassay kits from R&D Systems, (Minneapolis, MN) were used to determine serum concentrations of leptin, adiponectin, and IL-6. The ELISA assays employed the quantitative sandwich enzyme immunoassay technique using monoclonal

antibody pre-coated on 96-well micro plates for each cytokine. Samples were assayed in duplicate and compared to standard curves to determine serum activity. Absorbance was measured at specified wavelengths using a Molecular Devices Visible/UV plate reader with Soft Max computer software.

The leptin immunoassay is a 3.5 hour, solid-phase ELISA designed to measure soluble human leptin in cell culture supernates, serum, and plasma. The adiponectin and IL-6 immunoassays are 4.5 and 5.5 hour solid phase ELISA's respectively, also designed to measure human adiponectin and IL-6 in cell culture supernates, serum and plasma.

The coefficient of variation of duplicates (CV) and  $R^2$  value of linear regression were used to evaluate the strength of correlations of the measured and predicted results of each cytokine. An  $R^2$  value close to 1.0 indicates a very high correlation and reliability of sample results. The mean CV for leptin samples was 6.11% with a standard curve  $R^2$  of 0.99. The mean CV of adiponectin samples was 7.61% with a standard curve  $R^2$  of 0.99. The mean CV of IL-6 samples was 7.19% with a standard curve  $R^2$  of 0.985.

Mean serum leptin in a normal population is reported as 4760 pg/ml for males (range 2205-11149) and 20,676 pg/ml in females (range 3877-77,273) (R & D Systems, Inc., 2003). Leptin positively correlates with fat mass (Wang, Al-Regaiey, Masternak, & Bartke, 2006), BMI and body fat content (Anderlova et al., 2006).

Average plasma levels of adiponectin range from 5-10  $\mu\text{g/ml}$  and can range in non-obese subjects from 2-17  $\mu\text{g/ml}$  with plasma levels lower in men than women (Arita et al., 1999). Mean adiponectin expressed as ng/ml in normal population is 6641 ng/ml and can range from 865 – 21,424 ng/ml (R & D Systems, Inc., 2006). It parallels reduced insulin sensitivity in

obesity (Badman & Flier, 2005). Adiponectin is produced almost exclusively in adipose tissue and negatively regulated in obesity (Weyer et al., 2001).

Normal IL-6 levels in human lean subjects can range around  $0.39 \text{ pg/ml} \pm 0.06$ , to  $2.78 \text{ pg/ml} \pm 0.30$  in obese non-diabetic subjects, to as high as  $3.58 \text{ pg/ml} \pm 0.51$  in obese diabetic subjects (Bastard et al., 2000). One third of circulating IL-6 in obese humans comes from adipose tissue (Mohamed-Ali et al., 1997).

#### Data Collection and Procedure

Subjects were screened for inclusion and exclusion criteria. Subjects provided informed written consent (Appendix A). In addition, information was gathered regarding age, gender, ethnicity, menstrual status, physical activity level, current medications, and a 24 hour diet recall by self report using a questionnaire (Appendix B). A brief physical exam was conducted by a registered nurse researcher who measured height, weight, BMI, blood pressure (measured twice with subject seated), and resting apical pulse. Waist circumference was measured twice in a supine position. All data were collected on standardized forms throughout the study and verified for completeness by nurse researchers filling out the forms.

Subjects were asked to refrain from vigorous physical activity beyond their normal for the week prior to the initial face to face visit. Baseline blood samples (T1) were obtained after a 12 hour fast and drawn at Pathology Associates Medical Laboratory (PAML) by trained phlebotomists using standard technique. Following collection of baseline (T1) data, subjects followed a calorie restricted diet for 28 days. At the end of the 28 day diet, weight, resting blood pressure measured twice, and resting apical pulse was again taken. In addition, T2 lab samples for insulin, adiponectin, leptin and IL-6 were collected after a 12 hour fast at the same

PAML in the same time interval as above. Waist circumference was measured twice in a supine position. At the end of a 6-8 month post diet period (T3), the same data collected at T2 were repeated. Subjects were monitored weekly during the dietary intervention.

#### Calorie Restricted Diet

The subjects followed a 28 day calorie restricted diet following the collection of T1 baseline data, which equaled approximately 800 calories per day for women, and 1000 kcal per day for men. The diet consisted of commercially available, shakes/puddings, meal replacement bars, soups, oatmeal, yogurt and a portion of lean protein and green vegetables, divided into 6 small meals per day. Each meal consisted of approximately 90-140 calories.

Subjects kept daily records of the actual items eaten each day, including items not on the prescribed diet. They were coached on portion sizes for lean meats and vegetables. Most vegetables were allowed except carrots, peas, potatoes and corn. Non compliant subjects were encouraged to complete the diet and not dropped from the study. Researchers met with the subjects weekly. Weight was taken weekly, daily records reviewed, and BP and heart rate taken as described above.

Following the dietary intervention and collection of T2 data, subjects were asked if they could be contacted in 6-8 months time for follow up. Subjects were not encouraged to continue a caloric restriction, were free living, and self selected foods in the post diet period. When contacted in the 6-8 month post diet period T3, the same data collected at T2 was obtained with subject permission.

#### Data Analysis

Analysis of data was done using Statistical Package for the Social Sciences (SPSS) 14.0.

The level of significance for the repeated measured analysis was  $p < 0.05$ . Variables were analyzed for frequency, mean, standard deviation, and standard error mean. The T1 to T2, T2 to T3, and T1 to T3 comparisons were analyzed using a two tailed t test. The T1 to T2 and T2 to T3 paired samples were correlated for significance using Pearson's *r*. Bivariate correlations were also done using Spearman's rho on the T1 to T2, and T2 to T3 paired samples.

#### Data Management/Human Subjects Considerations

Procedures for data storage, entry, and cleaning followed guidelines for the larger parent study. All researchers completed a course in Human Participant Protections Education for Research provided by the National Cancer Institute and approved by Washington States University. The parent study was approved by the Washington State University Institutional Review Board (IRB) (Appendix C). This project was part of the parent study. HIPPA approval was not required as personal medical data was not used or generated in this study.

Data collected was held in strict confidentiality and security. Subjects were coded by a numbering system. The subject's names and study numbers were linked but kept separate. Access to data was limited to researchers involved in the study. The study data forms were kept in a separate locked file cabinet. The participants had all lab values made available to them at the conclusion of the study. Any critical lab values, if obtained, were followed up on, and the subject was encouraged to contact their primary care provider.

## CHAPTER THREE

### FINDINGS

#### Introduction

The purpose of this study was to evaluate the effects of a 28 day calorie restricted diet on serum measures of HOMA IR, leptin, adiponectin and IL-6 and weight. A repeated measures design was used in this study, part of the larger parent study investigation. Serum measures were taken before the 28 day calorie restricted diet (T1), after the calorie restricted diet intervention (T2), and in a 6-8 month post diet follow up period (T3).

#### Sample Characteristics

The larger parent study (n = 20) included 18 females and 2 males, with a mean age of 39.6 years, and were characterized as obese by BMI (mean = 36.2; SD = 6.3). One participant failed to provide a blood sample at T1. Of the 20 participants, 9 provided serum measures for the T1, T2 and T3 intervals. Data in the current study were analyzed using these 9 participants. See Table 3 for baseline characteristics of the larger study and the current study. Data were managed by entering information on a single computer with entries checked and rechecked by two separate researchers. Data were saved on the same computer and computer disks.

Analysis was performed by using SPSS version 14.0 software.

#### Statistical Analyses

Paired student's T-test was performed on all variables. T1 and T2, T2 and T3, and T1 and T3 differences were evaluated. Because of the small sample size (n = 9), both parametric and non parametric tests were done and the results compared. Pearson's *r* and Spearman's rho were performed on baseline data and on paired differences. Significant correlations were

reported for both tests. The Wilcoxon signed ranks test was performed on all variables for the T1 to T2, T2 to T3, and T1 to T3 changes and compared to the paired student's T- test and reported as a footnote. See Appendix D for scatter plots comparing selected variables. The student's T- test and Wilcoxon test displayed similar p values for the variables.

Table 3. Baseline Characteristics of Parent Study (n = 20) and Current Study (n = 9).

Characteristics	Mean	± SD	N	Characteristics	Mean	± SD	N
Age (years)	39.6	13.4	20	Age (years)	44.2	11.1	9
Weight (lbs)	223.1	53.8	20	Weight (lbs)	226.17	43.0	9
BMI (kg/m <sup>2</sup> )	36.2	6.3	19	BMI (kg/m <sup>2</sup> )	36.7	6.3	9
HOMA IR	4.6	2.6	19	HOMA IR	5.37	2.73	9
Leptin x 10 <sup>2</sup> pg/ml	442.73	208.25	19	Leptin x 10 <sup>2</sup> pg/ml	553.16	240.62	9
Adiponectin ng/ml	11207.63	7167.50	19	Adiponectin ng/ml	7205.94	4177.10	9
IL-6 pg/ml	2.50	1.79	19	IL-6 pg/ml	2.49	1.94	9

*Research Question #1*

Is there a significant correlation between weight, serum measurements of HOMA IR, IL-6, adiponectin and leptin at baseline?

The correlation between HOMA IR and adiponectin was significant using Pearson's *r* ( $r = -0.648$ ,  $p = 0.059$ ). There was no significant correlation using Pearson's *r* among the other variables. Spearman's rho however, demonstrated a significant negative correlation between HOMA IR and adiponectin ( $r = -0.667$ ,  $p = 0.050$ ), and weight and adiponectin ( $r = -0.700$ ,  $p = 0.036$ ). HOMA IR and IL-6 ( $r = 0.600$ ,  $p = 0.088$ ) did not reach significance. There were no other significant correlations between variables. See Table 4 for correlations of baseline data.

Table 4. Pearson's r and Spearman's rho Correlations of Baseline Data.

Pearson's r, 2-tailed significance at 0.05 level, n = 9					
	Weight T1	HOMA IR T1	Leptin T1	IL-6 T1	Adiponectin T1
Weight T1		r = 0.289 p = 0.451	r = -0.317 p = 0.406	r = -0.481 p = 0.190	r = -0.502 p = 0.169
HOMA IR T1	r = 0.217 p = 0.576		r = -0.212 p = 0.584	r = -0.358 p = 0.344	r = -0.648 p = 0.059
Leptin T1	r = -0.400 p = 0.286	r = -0.186 p = 0.637		r = -0.395 p = 0.293	r = 0.185 p = 0.634
IL-6 T1	r = -0.450 p = 0.224	r = 0.600 p = 0.088	r = 0.267 p = 0.488		r = -0.233 p = 0.546
Adiponectin T1	r = -0.700* p = 0.036	r = -0.667* p = 0.050	r = 0.017 p = 0.966	r = -0.367 p = 0.332	
*Spearman's rho, 2-tailed significance at 0.05 level, n = 9					

*Research Question # 2*

Is there a difference between weight, serum measurements of HOMA IR, IL-6, leptin and adiponectin collected at baseline (T1), after a 28 day 50% calorie restricted diet (T2), and at the end of a 6-8 month post diet period (T3)?

Nine participants provided serum measures for each of the three time intervals. See Table 5 for T1 and T2 comparison of variables. With the exception of IL-6, the mean changes for each variable reached significance. The mean weight loss between T1 and T2 was 14.82 pounds  $\pm$  4.87 SD,  $p < 0.001$ . Insulin sensitivity improved with weight loss. The mean HOMA IR decrease was 2.50  $\pm$  2.22 SD,  $p = 0.010$ . Leptin decreased a mean of 316.95  $\pm$  166.66 SD,  $p < 0.001$ . There was no significant change in serum IL-6 (0.45  $\pm$  1.99 SD,  $p = 0.520$ ). The change in adiponectin was a mean decrease of 797.43  $\pm$  1016.31,  $p = 0.046$ .



Table 5. Comparison of Weight, HOMA IR, Leptin, IL-6 and Adiponectin at T1 and T2.

Variable	Time 1 Mean ± SD	Time 2 Mean ± SD	Change Mean ± SD	t Score	df	p-value
Weight (lbs)	226 ± 43	211 ± 43	14.82 ± 4.87	9.135	8	< 0.001
HOMA IR	5.37 ± 2.73	2.87 ± 1.47	2.50 ± 2.22	3.380	8	0.010
IL-6 pg/ml	2.49 ± 1.94	2.05 ± 1.16	0.45 ± 1.99	0.673	8	0.520
Adiponectin ng/ml	7205.94 ± 4177.10	6409.51 ± 3365.33	797.43 ± 1016.31	2.354	8	0.046
Leptin x 10 <sup>2</sup> pg/ml	553.16 ± 240.62	236.21 ± 150.11	316.95 ± 166.66	5.705	8	< 0.001

Wilcoxon signed rank test for T1 to T2. Weight and leptin, p = 0.008; HOMA IR, p = 0.011; Adiponectin, p = 0.051; IL-6, p = 0.594

Differences between the T2 and T3 measures are shown in Table 6. Only weight, HOMA IR and leptin changes reached significance. Weight (lbs) increased a mean of 13.68 ± 10.09 SD, p = 0.004. Insulin sensitivity decreased with a HOMA IR mean increase of 1.82 ± 2.07 SD, p = 0.030. There was no significant change in IL-6, (mean -0.13 ± .84 SD, p = 0.652). Leptin had a mean increase of 309.14 ± 177.74, p = 0.001. There was a nonsignificant increase in Adiponectin, (mean of 347.30 ± 1393.82, p = 0.476).

Table 6. Comparison of Weight, HOMA IR, Leptin, IL-6 and Adiponectin at T2 and T3.

Variable	Time 2 Mean ± SD	Time 3 Mean ± SD	Change Mean ± SD	t Score	df	p-value
Weight (lbs)	211 ± 43	225 ± 47	13.68 ± 10.1	4.072	8	0.004
HOMA IR	2.87 ± 1.47	4.70 ± 3.04	1.82 ± 2.07	2.638	8	0.030
IL-6 pg/ml	2.05 ± 1.16	1.91 ± 0.90	0.13 ± 0.84	0.468	8	0.652
Adiponectin ng/ml	6408.51 ± 3365.33	6755.81 ± 3600.64	347.30 ± 1393.82	0.748	8	0.476
Leptin x 10 <sup>2</sup> pg/ml	236.21 ± 150.11	545.35 ± 245.64	309.14 ± 177.74	5.22	8	0.001

Wilcoxon signed rank test for T2 to T3. Weight and leptin,  $p = 0.008$ ; HOMA IR,  $p = 0.038$ ; IL-6,  $p = 0.594$ ; Adiponectin,  $p = 0.515$ .

A comparison of T1 and T3 reveals that the nine subjects essentially returned to baseline for all the measured parameters with the exception of IL 6 which did not change significantly at any point. None of the T1 to T3 differences reached significance. See Table 7 for comparisons between T1 and T3. Weight (lbs) showed a mean difference of  $-1.13 \pm 8.16$  SD,  $p = 0.688$ . Insulin sensitivity was only slightly improved compared to baseline. HOMA IR had a mean decrease of  $0.67 \pm 2.14$  SD,  $p = 0.373$ . Adiponectin showed a mean difference compared to baseline of  $-450.13 \pm 1531.65$  SD,  $p = 0.404$ . Leptin also increased at T3 with a mean difference compared to baseline of  $7.81 \pm 198.63$  SD,  $p = 0.909$ .

Table 7. Comparison of Weight, HOMA IR, Leptin, IL-6 and Adiponectin at T1 and T3.

Variable	Time 1 Mean ± SD	Time 3 Mean ± SD	Change Mean ± SD	t Score	df	p-value
Weight (lbs)	226 ± 43	225 ± 49	1.13 ± 8.16	0.416	8	0.688
HOMA IR	5.37 ± 2.73	4.70 ± 3.04	0.67 ± 2.14	0.943	8	0.373
IL-6 pg/ml	2.49 ± 1.94	1.91 ± 0.90	0.58 ± 1.60	1.085	8	0.310
Adiponectin ng/ml	7205.94 ± 4177.10	6755.81 ± 3600.64	450.13 ± 1531.65	0.882	8	0.404
Leptin x 10 <sup>2</sup> pg/ml	553.16 ± 240.62	545.35 ± 245.64	7.81 ± 198.63	0.118	8	0.909

Wilcoxon signed rank test for T1 to T3. Weight, p = 0.767; HOMA IR, p = 0.260; IL-6, p = 0.515; Adiponectin, p = 0.374; Leptin, p = 0.953

*Research Question #3*

Is there a relationship between change scores in weight, insulin sensitivity, IL-6, leptin and adiponectin?

Of all the variables, only weight and adiponectin change scores from T1 and T2 had a significant negative correlation ( $r = -0.703$ ,  $p = 0.035$ ) with Pearson's  $r$ . Spearman's rho showed a negative correlation between weight and adiponectin ( $r = -0.756$ ,  $p = 0.018$ ) change scores from T1 to T2. None of the other variables had change scores that reached significance. See Table 8 for Pearson's  $r$  and Spearman's rho correlation comparisons.

Table 8. Pearson's *r* and Spearman's rho Change Scores Correlations T1 to T2.

*Pearson's <i>r</i> , 2-tailed significance at 0.05 level, n = 9					
	Weight T1 to T2	HOMA IR T1 to T2	Leptin T1 to T2	IL-6 T1 to T2	Adiponectin T1 to T2
Weight T1 to T2		<i>r</i> = 0.107 <i>p</i> = 0.783	<i>r</i> = -0.557 <i>p</i> = 0.120	<i>r</i> = -0.410 <i>p</i> = 0.273	<i>r</i> = -0.703* <i>p</i> = 0.035
HOMA IR T1 to T2	<i>r</i> = 0.244 <i>p</i> = 0.527		<i>r</i> = -0.216 <i>p</i> = 0.576	<i>r</i> = -0.317 <i>p</i> = 0.406	<i>r</i> = -0.287 <i>p</i> = 0.454
Leptin T1 to T2	<i>r</i> = -0.345 <i>p</i> = 0.362	<i>r</i> = -0.200 <i>p</i> = 0.606		<i>r</i> = -0.215 <i>p</i> = 0.579	<i>r</i> = 0.391 <i>p</i> = 0.298
IL-6 T1 to T2	<i>r</i> = -0.311 <i>p</i> = 0.415	<i>r</i> = 0.367 <i>p</i> = 0.332	<i>r</i> = -0.550 <i>p</i> = 0.125		<i>r</i> = -0.107 <i>p</i> = 0.783
Adiponectin T1 to T2	<i>r</i> = -0.756* <i>p</i> = 0.018	<i>r</i> = -0.317 <i>p</i> = 0.406	<i>r</i> = 0.267 <i>p</i> = 0.488	<i>r</i> = 0.033 <i>p</i> = 0.932	
*Spearman's rho, 2-tailed significance at 0.05 level, n = 9					

*Research Question #4*

Is there a relationship between change scores in weight, HOMA IR, IL-6, leptin and adiponectin after calorie restriction and at the end of the 6-8 month post diet period?

Pearson's *r* showed no significant change score correlations between variables comparing T2 and T3, though HOMA IR and weight was trending in the direction of significance (*r* = 0.602, *p* = 0.086). Spearman's rho demonstrated no significant change score correlations between variables comparing T2 to T3. See Table 9 for Pearson's *r* and Spearman's rho change score correlations T2 to T3.

Table 9. Pearson's r and Spearman's rho Change Scores Correlations T2 to T3.

Pearson's r, 2-tailed significance, n = 9					
	Weight T2 to T3	HOMA IR T2 to T3	Leptin T2 to T3	IL-6 T2 to T3	Adiponectin T2 to T3
Weight T2 to T3		r = 0.602 p = 0.086	r = 0.000 p = 1.000	r = -0.223 p = 0.565	r = -0.056 p = 0.885
HOMA IR T2 to T3	r = 0.410 p = 0.273		r = -0.341 p = 0.370	r = 0.393 p = 0.295	r = -0.395 p = 0.293
Leptin T2 to T3	r = 0.126 p = 0.748	r = -0.167 p = 0.668		r = -0.318 p = 0.404	r = 0.375 p = 0.320
IL-6 T2 to T3	r = -0.418 p = 0.262	r = 0.400 p = 0.286	r = -0.333 p = 0.381		r = -0.326 p = 0.393
Adiponectin T2 to T3	r = 0.084 p = 0.831	r = -0.550 p = 0.125	r = 0.250 p = 0.516	r = -0.417 p = 0.265	
Spearman's rho, 2-tailed significance, n = 9					

### Summary

The relationship between weight, serum measurements of HOMA IR, leptin, IL-6 and adiponectin in individuals, overweight and at risk for diabetes was evaluated in this study. All data collected at baseline were part of a larger research study involving 20 individuals. Of the study participants, 9 individuals provided data at baseline (T1), after a 28-day calorie-restricted dietary intervention (T2), and after a 6-8 month post diet period (T3).

The findings show that the differences between T1 and T2 were significant for weight, HOMA IR, adiponectin and leptin but not IL-6. Overall the subjects essentially returned to baseline measures in the 6-8 month post diet period. The differences from T2 to T3 were again significant for weight, HOMA IR, and leptin but not IL-6 or adiponectin. IL-6 decreased from T1

to T2 to T3 but the changes did not reach significance. Comparison of T1 and T3 showed no significance for any variable, as every variable, apart from IL-6, essentially returned to baseline.

Adiponectin correlated negatively with weight and HOMA IR at baseline. T1 and T2 change scores revealed a significant negative correlation between adiponectin and weight.

## CHAPTER 4

### SUMMARY, CONCLUSIONS, RECOMMENDATIONS

#### Introduction

The relationships between weight, HOMA IR, adiponectin, leptin, and IL-6, in overweight and obese persons ( $\text{BMI} \geq 26 \text{ kg/m}^2$ ) at risk for type 2 diabetes, were investigated in this study. Based on systems theory, it was hypothesized that changes in one component of a system affects other components in the system. Hence, it was predicted that there would be significant relationships between the different variables investigated as well as between the time comparisons.

Baseline data were collected as part of a larger research study. The larger study had a sample of 20 subjects. Of the twenty subjects, nine provided data for three time intervals, at baseline T1, after a 28 day calorie restriction diet T2, and at the end of a 6-8 month post diet period T3.

#### Discussion

The current study revealed a number of significant correlations. Comparing baseline variables, adiponectin was negatively correlated with weight ( $r = -0.70$ ,  $p = 0.036$ ) and HOMA IR ( $r = -0.667$ ,  $p = 0.050$ ). HOMA IR was trending, but did not reach significance with IL-6 ( $r = 0.60$ ,  $p = 0.088$ ).

T1 to T2 change scores showed a negative correlation between weight and adiponectin ( $r = -0.703$ ,  $p = 0.035$ , Pearson correlation) and ( $r = -0.756$ ,  $p = 0.18$ , Spearman's rho). T2 to T3 change scores revealed no significant correlations. HOMA IR was trending, but did not reach significance with weight ( $r = 0.602$ ,  $p = 0.086$ ).

T1 to T2, comparisons were significant for decreased weight and leptin ( $p < 0.001$ ), adiponectin ( $p = 0.046$ ), and HOMA IR ( $p = 0.010$ ). There was no significant change in IL-6 ( $p = 0.520$ ). T2 to T3 comparisons were significant for increased weight ( $p = 0.004$ ), HOMA IR ( $p = 0.030$ ), leptin ( $p = 0.001$ ). There was no significant change in IL-6 ( $p = 0.652$ ). There was a nonsignificant increase in adiponectin ( $p = 0.476$ ). T1 to T3 comparisons did not reach significance for any variable.

Baseline correlation of adiponectin and weight was significant in the current study ( $r = -0.70$ ,  $p = 0.036$ ). A similar finding was noted by Vendrell and colleagues (2004) ( $r = -0.33$ ,  $p = 0.001$ ) correlating baseline variables in a nonmorbidly obese control group of subjects (BMI 32 kg/m<sup>2</sup>), but not in correlation of baseline variables in a morbidly obese intervention group (BMI 49.3 kg/m<sup>2</sup>) (no  $p$  value reported). The nonmorbidly obese group has a similar BMI to the baseline sample in the present study (36.7 kg/m<sup>2</sup>).

The baseline correlation of adiponectin and HOMA IR ( $r = -0.667$ ,  $p = 0.50$ ) was congruent with other studies (Xydakis et al., 2004; Arita et al., 1999; Mojiminiyi, Abdella, Arouji, & Nakhi, 2007). The current study found a trend toward significance in baseline correlation of HOMA IR and IL-6 ( $r = 0.60$ ,  $p = 0.088$ ). Bruun, et al. (2003) reported a significant correlation between baseline HOMA IR and IL-6 ( $r = 0.57$ ,  $p < 0.01$ ).

The present study found significant correlations for change scores from T1 to T2 for weight and adiponectin. No other studies reviewed reported significant correlations for change scores between weight and adiponectin. Vendrell, et al. (2004), analyzing whether hormone tissue changes were predictive of insulin sensitivity, reported a significant correlation between



HOMA IR and adiponectin ( $\beta = 0.77$ ,  $p = 0.014$ ). The present study showed no significant correlation between HOMA IR and adiponectin change scores ( $r = -0.287$ ,  $p = 0.454$ ).

The study by Vendrell and coworkers (2004) noted the significant change in morbidly obese subjects after gastric bypass surgery. There was not a significant relationship in the morbidly obese group in baseline correlation of HOMA IR and adiponectin. In explaining the findings, Vendrell, et al. (2004) suggest the post surgical, massive weight loss in this morbidly obese cohort of subject may reflect marked changes in proinflammatory cytokines, and increased plasma levels of tissue metabolism modulators such as adiponectin. The resulting adiponectin increase was predictive of changes in HOMA IR (Vendrell et al., 2004), supporting the notion that normal endocrine loops may somehow be lost in morbid obesity (Vendrell et al., 2004).

Other studies did confirm some of the T1 to T2 comparisons found in the current study. The significant decrease in weight in the current study was a similar finding in other studies (Xydakis et al., 2004; Bastard et al., 2000; Bruun et al., 2003; Vendrell et al., 2004; Weiss et al., 2006; Salas-Salvado et al., 2006). A similar significant decrease in leptin was also noted (Xydakis et al., 2004; Bastard et al., 2000; Gallistl et al., 2001; Klein et al., 2004; Vendrell et al., 2004).

A significant decrease in HOMA IR or other measurement of insulin sensitivity was a significant finding noted by other researchers (Xydakis et al., 2004; Weiss et al., 2006; Bastard et al., 2000; Bruun et al., 2003; Salas-Salvado et al., 2006; Giugliano et al., 2004; Vendrell et al., 2004; Ross et al., 2000). Ross, et al. (2004) in their study of obese women, unexpectedly found no significant decrease in insulin sensitivity in a calorie restriction intervention group, but did in the study of obese men (Ross et al., 2000) that mirrors the study design of the obese women.

Adam et al. (2006) noted no change between T1 and T2 for HOMA IR in their study of glucagon-like peptide 1 before, and after a 6 week diet induced weight loss (Adam et al., 2006).

In the current study, decreases in IL-6 between T1 and T2 did not reach significance. Other studies however, reported a significant IL-6 decrease between T1 and T2 (Bastard et al., 2000; Bruun et al., 2003; Gallistl et al., 2001; Giugliano et al., 2004; Vendrell et al., 2004). Possible explanations are that the sample size was too small to capture significant changes or the changes may reflect some sort of metabolic dysregulation.

Various studies reported conflicting results for adiponectin changes from baseline (T1) to post-weight loss (T2). The current study found a significant decrease in adiponectin ( $p = 0.046$ ). Xydakis and coworkers (2004) found no significant change in adiponectin ( $p = 0.10$ ) though levels decreased. Two studies reported significant increases (Giugliano et al., 2004; Vendrell et al., 2004). Weiss and colleagues (2006) noted an increase in adiponectin that did not reach significance ( $p = 0.07$ ).

Weiss and colleagues (2006) offer a possible explanation for the seemingly conflicting results observed with adiponectin. Adiponectin is noted to increase in weight loss induced by medication use (Valsamakis et al., 2004), or after intestinal bypass surgery (Vendrell et al., 2004). Adiponectin does not typically increase after weight loss induced by diet or weight loss training (Bastard et al., 2000). Based on cross sectional data, Weiss and colleagues (2006) noted that in studies where adiponectin did not increase, obese individuals were being studied (Weiss et al., 2006). In their study, Weiss and colleagues (2006) noted an increase in adiponectin that did not reach significance ( $p=0.70$ ), but the study involved overweight ( $BMI < 30 \text{ kg/m}^2$ )

individuals. Weiss and colleagues (2006) observed that adiponectin is associated with BMI, but only when BMI is below 29 kg/m<sup>2</sup> (Arita et al., 1999; Weiss et al., 2006).

The current study noted a significant decrease in adiponectin in an overweight and obese cohort of subjects (BMI 36.7 kg/m<sup>2</sup>). However, the current study did not correlate BMI with other variables studied. Adiponectin changes in the current study may reflect the explanation proposed by Weiss and colleagues (2006). Of the two studies that reported significant increased adiponectin levels, one was 3 months after liposuction while in weight maintenance (Giugliano et al., 2004), and the other was 6 months after gastric bypass surgery, though it is not clear from the study if subjects were in a weight maintenance state at the time of the 6 month measures (Vendrell et al., 2004).

There are limited studies to compare the current study T2 to T3 comparisons. One study by Adam and colleagues (2006), measured levels of Glucagon-like peptide 1 (GLP-1) before a weight loss intervention, and after a 6 week diet weight loss intervention, and in a 3 month post diet weight maintenance state. HOMA IR was measured at baseline and at the end of the diet intervention with no change noted. No measurement was done of HOMA IR at the end of the 3 month weight maintenance state, but researchers noted an unexpected rebound of GLP 1 (Adam, Lejeune, & Westerterp-Plantenga, 2006).

A study by Salas-Salvado and coworkers (2006) measured HOMA IR in 19 morbidly obese subjects at baseline (T1), after a 6-week calorie restricted diet intervention (T2), and after a two week weight maintenance phase (T3). Subjects maintained weight loss in the two weeks following the calorie restriction. HOMA IR decreased significantly from T1 to T2 ( $p <$

0.001) with weight loss, but increased significantly T2 to T3 ( $p < 0.05$ ) while weight loss was maintained (Salas-Salvado et al., 2006).

By comparison, the current study noted a significant T2 to T3 increase in HOMA IR ( $p = 0.030$ ) along with a significant increase in weight ( $p = 0.004$ ).

The current study found no significant differences in the T1 to T3 comparisons, as subjects essentially returned to baseline in the 6-8 month post diet period, with the exception of IL-6, which continued to decrease throughout the three time periods, but changes did not reach statistical significance.

In summary, findings of the current study support, in part, the hypothesized predictions in the systems theory model. HOMA IR and Leptin decreased with weight loss, and increased with weight gain. Adiponectin was predicted to increase with weight loss and decrease with weight gain, but the converse occurred. Adiponectin decreased from T1 to T2 and increased from T2 to T3 concomitantly with the decrease and increase of weight, HOMA IR and Leptin over the same time intervals. IL-6 decreased from T1 to T2 as predicted, but did not increase as predicted from T2 to T3. The pattern adiponectin followed may reflect the explanation offered by Weiss and coworkers (2006), reconciling varied findings of other reported studies. Results may also reflect measurement in a negative energy state at T2. The changes in IL-6 may reflect metabolic dysregulation.

Although the T1 to T2 comparisons were similar to other study results, findings reflect those taken in a calorie restricted state, and observed changes were not sustained in a post diet period. A number of researchers suggest the need for studies that include a post diet period (Bastard et al., 2000; Xydakis et al., 2004; Gallistl et al., 2001; Wadden, Anderson, & Foster,

1999) to see if changes measured in a calorie restricted state continue long term for subjects who maintain weight loss, and for subjects who regain weight.

The lack of studies including a post diet period following a calorie restricted diet intervention limit meaningful comparisons across time, and especially in the post-diet phase.

#### Limitations

This pilot study was limited by its small sample size ( $n = 9$ ). The small numbers may contribute to some correlations approaching, but not reaching statistical significance. Conversely, there is the danger of making a type 1 statistical error, where a positive relationship may seem to exist, that may not be true in a larger sample size (Polit & Beck, 2004).

It is not uncommon in this type of dietary intervention study to have a high number of subjects who fail to complete the diet phase, or fail to complete the post diet phase follow up. In addition, keeping free living human subjects adherent to dietary protocols is difficult even with weekly follow up, as was done in the current study during the dietary intervention.

Because the study consisted of a small convenience sample of individuals at risk for type 2 diabetes, it is not possible to generalize results to the larger population. In addition, subject participation was limited to those with a BMI of  $\geq 26 \text{ kg/m}^2$ , which may not reflect the general population. The variables investigated in this study can vary based on the level of obesity (Vendrell et al., 2004; Bastard et al., 2000) or the presence of metabolic syndrome (Xydakis et al., 2004). In addition, the current study did not take into account ethnic differences and variability that are possible among cytokines and other variables studied in this research (Nakai et al., 2002; Weyer et al., 2001).

The current study is unique in that it collected data in a 6-8 month post diet period. Only one study was found that had a 3 month post diet intervention weight maintenance period (Adam et al., 2006), hence, it was not possible to compare results collected at T3 meaningfully to other studies. The current study participants, for the most part, returned to their baseline status in the 6-8 month post diet period, which further limits meaningful comparison. There is a paucity of research that measures data in a weight maintenance state after a calorie restricted diet intervention.

The current study compared baseline measures (T1), to measures taken in a calorie restricted state (T2). The effects of calorie restriction on the various cytokines are not completely understood. Changes measured may reflect some form of metabolic dysregulation induced by the calorie restriction, (Xydakis et al., 2004). Some suggest that a negative energy state, such as that found after weight loss or calorie restriction, may in short term, promote expression of adipocytokines such as adiponectin, which stimulate the mobilization of fat stores in calorie restriction (Salas-Salvado et al., 2006) or massive weight loss (Vendrell et al., 2004), while at the same time reduce acute phase reactants and pro-inflammatory cytokines (Salas-Salvado et al., 2006).

In a weight stabilization period, cytokine over expression involved in modulating and mobilizing fat stores, may be restored or reduced, and inflammation levels may return to baseline (Salas-Salvado et al., 2006). This may account for some disparity of results seen in the literature, and may be reflective of the energy balance state in which the measures were taken (Salas-Salvado et al., 2006). It illustrates the need for studies to include an isocaloric or weight stable period following a calorie restricted diet or weight loss intervention, to see if changes

observed in a calorie restricted or weight loss state persist over time (Xydakis et al., 2004; Gallistl et al., 2001). Because individuals in the present study regained weight that was, for the most part, equivalent to baseline weight, this study does not contribute to knowledge about HOMA IR and cytokine activity in weight-stable individuals following a calorie restricted diet.

### Implications

It is common for individuals who are overweight and obese to regain weight after weight loss. This occurred in nearly all of the subjects involved in the current study. The benefit gained in the short term weight loss was erased in the post diet period. There is a need for long term effective strategies to attain and maintain weight reduction. Short term weight reduction may not be an effective strategy, even in the face of positive significant findings from the present study. There is literature that supports the notion that weight cycling is more detrimental to the health of overweight individuals, than maintaining a stable weight over time (Jefferey, 1996; Truesdale, Stevens, Schreiner, Loria, & Cai, 2006; Hamm, Shekelle, & Stamler, 1989; Lissner et al., 1991; Lee & Paffenbarger, 1992; Rodin, Radke-Sharpe, Rebuffe-Scrive, & Greenwood, 1990).

The present study did find significant changes, comparing baseline (T1), to measures taken after a calorie restricted diet intervention (T2), in weight, HOMA IR and leptin that was congruent with previous studies. These changes support those predicted in the systems theory model. However, changes in adiponectin, though significant, did not reflect those predicted in the model. This highlights the need for more research to understand the interactions of the various cytokines in obesity. Research findings may result in more effective long term treatment strategies for obesity.

## Recommendations

The current pilot study used a repeated measures design to examine relationships and differences between changes in weight, HOMA IR, leptin, adiponectin and IL-6 in a specific overweight and obese patient population of individuals at risk for type 2 diabetes. A larger study, involving more subjects, compared to a normal weight control group is needed to confirm the significant changes observed in this study. Including a weight maintenance component in future studies would enable researchers to meaningfully compare results of measures taken in a calorie restricted state to those obtained following a calorie restricted diet intervention.

To illustrate, one prospective study by Wadden and colleagues (1999) followed 25 obese women who had lost  $\geq 5\%$  of initial weight over a 48 week period, and maintained the weight at one year follow up. Using serial measures at week 8, 24, 48 and week 100, HDL cholesterol declined significantly at week 8 ( $p < 0.05$ ), but returned to baseline by week 24. There was some weight gain from week 48 to week 100 which caused LDL cholesterol to rise significantly ( $p < 0.05$ ). Patients who at week 100 maintained  $> 10\%$  loss of initial weight had significantly greater reductions in LDL cholesterol ( $p < 0.05$ ) values than patients who maintained losses of only  $5\%$  to  $10\%$  of initial weight (Wadden et al., 1999).

Studies, like the one illustrated, that also measured cytokine activity, would better enable clinicians to counsel and guide patients with more effective long term strategies. Studies measuring the post short and long term effects of a weight loss or a dietary intervention, would add meaningfully to the current, but growing understanding of adipose tissue as an endocrine organ. As the roles and interactions between the various cytokines are better defined, an



increased understanding of the complex pathophysiology of obesity and its relationship to type 2 diabetes, the metabolic syndrome, and insulin resistance, will enable clinicians to be better equipped to treat patients at risk for type 2 diabetes who are overweight or obese.

### Conclusion

Obesity is a worldwide problem with deleterious effects on the health of individuals and increasing the economic costs of healthcare. This study found significant improvements in weight, leptin, adiponectin, and HOMA IR following a calorie restricted diet intervention in an overweight and obese population of individuals at risk for type 2 diabetes. A significant positive correlation was found between weight and adiponectin following the calorie restricted diet intervention but not between other variables studied. The results, in part, agree with the predictions hypothesized in the systems theory model.

However, subjects, for the most part, regained the weight lost, and HOMA IR and the cytokines measures returned to baseline in the 6-8 month post diet period. These findings suggest that although short-term calorie restriction achieves significant weight loss and changes in HOMA IR and some cytokines, the changes are not sustained. It is unclear if weight reaccumulation causes the cytokines and HOMA IR to return to baseline or if cytokine dysregulation along with insulin resistance cause weight to return to baseline. Perhaps there are other factors affecting both parameters. Further study is needed to understand these relationships. Based on the findings of this study, short term calorie restricted diets to achieve weight loss cannot be recommended as an effective strategy for an individual at risk for developing type 2 diabetes. A more effective, successful, long term strategy should be recommended.

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## Appendix A

### WASHINGTON STATE UNIVERSITY

### CONSENT FORM

#### **The Effect of Diet-Induced Weight Loss on Abdominal Fat and Metabolic Profile In Men and Women At Risk for Diabetes**

Researcher: **Jacquelyn Banasik**, PhD, ARNP **Phone:** 324-7269 **Email:** banasik@wsu.edu

#### Researcher's statement

You are being invited to participate in a research study. The purpose of this consent form is to give you the information you will need to help you decide whether to be in the study or not. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When all your questions have been answered, you can decide if you want to be in the study or not. This process is called 'informed consent.' A copy of this form will be given to you for your records.

#### PURPOSE AND BENEFITS

The purposes of this project are 1) to describe the relationship between abdominal cavity fat mass and certain hormones and metabolic indicators that may contribute to weight gain and diabetes risk; and 2) to determine the short and long-term effects of a low-calorie diet-induced weight reduction on abdominal fat mass in persons at risk for type 2 diabetes.

#### PROCEDURES

After screening to make sure you fit the inclusion criteria of having a first degree relative with type 2 diabetes or a body mass index greater than 26 kg/m<sup>2</sup>, you will be asked to abstain from vigorous exercise, then fast for 12 hours before having your blood drawn from a vein (about 1 1/2 tablespoons) and to have your height, weight, blood pressure, heart rate, and waist circumference measured. You will be asked to recall your dietary intake over the past 24 hours and to answer questions about your family medical history, medications you may be taking and any medical problems you may have. You will be asked to fill out questionnaires concerning diet history, mood, stress, and psychosocial factors. You will be asked to collect saliva samples four times per day for two consecutive days and you will be scheduled for an appointment to have a CT scan of your abdomen at a local CT scan facility. The initial visit will take approximately 90 minutes to complete. The CT scan will take only 5 minutes, however you may need to wait for your appointment for up to 15 minutes if the facility is busy. You will then follow a normalized diet for 3 days and then have another blood sample taken.

You will then enter the intervention phase during which you will be asked to follow a strict low-calorie diet of approximately 800 calories per day for up to 28 days or until you achieve a weight loss of 8% to 10% of your initial body weight. The diet will consist of approximately 500 calories of commercially available items including diet shakes, bars, soups, and cereals which will be

provided to you in weekly allotments. You will also be asked to consume a 4 oz portion of lean meat or protein plus a green salad or vegetable portion each day equal to about 300 calories. The meat and vegetables must be purchased by you. All other food items will be provided. You will be asked to record the actual food items eaten each day during the diet and to visit the HSB weekly for weight measures and to receive the weekly dietary allotment. At the end of the diet intervention, and after a 12 hour fast, you will again be asked to complete questionnaires, have body measures taken, a CT scan performed, blood drawn, and complete saliva collections. After completion of this part of the study you will be asked to follow a weight maintenance diet for 2 weeks. Another blood sample will be taken at the end of this 2 week period. You will then be contacted 10 to 12 months later to again complete questionnaires, have body measures taken, have a CT scan performed, blood drawn and collect saliva samples. During the study, blood will be drawn on 5 occasions for a total of 7.5 tablespoons.

### RISKS, STRESS, OR DISCOMFORT

Potential risks from participating in this study include infection, minor pain, or bruising at the skin where blood is drawn. All blood samples will be obtained by trained personnel using standard infection prevention technique. Risks from dieting include hunger, fatigue, light-headedness, and constipation. A nurse practitioner will be available to help you manage any problems that arise because of dieting. Although you are encouraged to follow the diet as prescribed, you have control over your dietary intake at all times. Abdominal fat will be measured by CT scan. The scan gives minimal radiation exposure similar to that of a chest film. Women must not become pregnant during the study because of the risks of dietary restrictions and radiation exposure to the fetus. There is a possibility that you could see other participants in the research areas and they may be aware of your involvement in the study. Your personal data will be kept confidential at all times.

### OTHER INFORMATION

Data collected during the study will be confidential and available only to the investigators. No identifying data will be included in grants, journal publications, or thesis manuscripts that result from this research. Data will be stored in locked files in the investigators office and destroyed after 3 years. You may refuse to participate or you may withdraw from the study at any time without penalty. Women must not become pregnant during the study. You will receive no compensation for your participation. You will be expected to purchase your own dietary meats and greens. You will not be responsible for any other costs associated with the study. If you are injured as a direct result of study procedures, you will be evaluated by a nurse practitioner on the research team and referred to your primary care provider for appropriate treatment if necessary. You will be responsible for the costs of evaluation and treatment by your health care provider.

### MEDICAL RECORDS ACCESS

No medical record review will be done. All information will be collected directly from you.

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Jacquelyn Banasik, PhD, ARNP

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Printed name of researcher

Signature of researcher

Date

Subject's statement

This study has been explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have general questions about the research, I can ask the researcher listed above. If I have questions regarding my rights as a participant, I can call the WSU Institutional Review Board at (509)335-9661. This project has been reviewed and approved for human participation by the WSU IRB. I will receive a copy of this consent form.

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Printed name of subject

Signature of subject

Date

Appendix B

Demographics and Weight Cycling Questionnaire

Subject Number \_\_\_\_\_ Birth Date \_\_\_\_\_ Today's Date \_\_\_\_\_

Do you smoke? Yes \_\_\_ No \_\_\_

Gender: 1. Male \_\_\_ 2. Female \_\_\_

Age \_\_\_\_\_ BP \_\_\_\_\_ BP \_\_\_\_\_ Heart Rate \_\_\_\_\_  
Current Weight \_\_\_\_\_ # Height \_\_\_\_\_ inches

BMI \_\_\_\_\_ Waist circumference \_\_\_\_\_ cm \_\_\_\_\_ cm

Family History:

1. Type-2 Diabetes \_\_\_\_\_
2. Obesity \_\_\_\_\_
3. Hypertension \_\_\_\_\_

Do you exercise regularly? Yes \_\_\_ No \_\_\_ If yes, please describe \_\_\_\_\_

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How many pregnancies have you had:

0 \_\_\_ 1 \_\_\_ 2 \_\_\_ 3 \_\_\_ 4 \_\_\_ 5 \_\_\_ 6 \_\_\_ 7 \_\_\_ 8 \_\_\_ 9 \_\_\_ 10+ \_\_\_

What was your weight gain with pregnancy?

1 \_\_\_ 2 \_\_\_ 3 \_\_\_ 4 \_\_\_ 5 \_\_\_ 6 \_\_\_ 7 \_\_\_ 8 \_\_\_ 9 \_\_\_ 10 \_\_\_

Are you postmenopausal (no periods for greater than a year.)? 1.Yes \_\_\_ 2.No \_\_\_

What was your weight at age 18? \_\_\_\_\_

What was your lightest weight after the age of 18? \_\_\_\_\_

What was your heaviest weight after the age of 18? \_\_\_\_\_

What was your lightest weight in your:

1. 20's \_\_\_\_\_ 2. 30's \_\_\_\_\_ 3. 40's \_\_\_\_\_ 4. 50's \_\_\_\_\_ 5. 60's \_\_\_\_\_

What was your heaviest weight in your:

1. 20's \_\_\_\_\_ 2. 30's \_\_\_\_\_ 3. 40's \_\_\_\_\_ 4. 50's \_\_\_\_\_ 5. 60's \_\_\_\_\_

Not including delivery of babies, how many times in your lifetime have you experienced weight loss of at least:

1. 10-19 pounds \_\_\_\_\_
2. 20-29 pounds \_\_\_\_\_
3. 30-39 pounds \_\_\_\_\_
4. 40-49 pounds \_\_\_\_\_
5. >50 pounds \_\_\_\_\_

In the last 10 years how many times have you attempted to lose weight using a calorie restricted diet which you followed for at least two weeks?

0 – 5 times \_\_\_\_\_

6 to 10 times \_\_\_\_\_

11 to 20 times \_\_\_\_\_

more than 20 times \_\_\_\_\_

Please list any food allergies: \_\_\_\_\_

Please list any food dislikes: \_\_\_\_\_

Please list all medications you are taking:

- |          |           |
|----------|-----------|
| 1. _____ | 7. _____  |
| 2. _____ | 8. _____  |
| 3. _____ | 9. _____  |
| 4. _____ | 10. _____ |
| 5. _____ | 11. _____ |
| 6. _____ | 12. _____ |

Do you have any of the following conditions (check all that apply):

- |                               |                              |
|-------------------------------|------------------------------|
| 1. Asthma _____               | 8. Lupus _____               |
| 2. Cancer _____               | 9. Crohns _____              |
| 3. COPD _____                 | 10. Ulcerative colitis _____ |
| 4. Hypothyroid _____          | 11. Hypertension _____       |
| 5. Hyperthyroid _____         | 12. High Cholesterol _____   |
| 6. CHF _____                  | 13. Other _____              |
| 7. Rheumatoid Arthritis _____ |                              |

Race/Ethnicity (check only one):

1. American Indian or Alaskan Native \_\_\_\_\_
2. Asian American \_\_\_\_\_
3. Black or African American \_\_\_\_\_
4. Hispanic or Latino \_\_\_\_\_
5. Native Hawaiian or Pacific Islander \_\_\_\_\_
6. White \_\_\_\_\_
7. Multiracial (two or more of the above categories) \_\_\_\_\_

24-hour Diet Recall. List all foods eaten the previous day. Include portion sizes and brand names if known.



## Appendix C



Research Compliance Office

### MEMORANDUM

**TO:** Jacquelyn Banasik  
Intercollegiate College of Nursing, Spokane

**FROM:** Malathi Jandhyala (for) Kris Miller, Chair, WSU Institutional Review Board *AKM*

**DATE:** 12 September 2006

**SUBJECT:** Review of Protocol Modification - Modification

Your proposal to modify the protocol titled *"Effect of Diet-Induced Weight Fluctuation on Visceral Adiposity and Metabolic Profile in Men and Women at risk for Type 2 Diabetes,"* IRB File Number 8945-b was reviewed for the protection of the subjects participating in the study. Based on the information received from you, the IRB has **approved** your modification request on **12 September 2006**. This modification includes a reduction of sample size, saliva sample instead of urine sample, and elimination of BODPOD fat assessment.

IRB approval indicates that the modifications described to the previously approved study protocol are designed to adequately protect the subjects participating in the study. This approval does not relieve the investigator from the responsibility of providing continuing attention to ethical considerations involved in the utilization of subjects participating in the study.

**The approval for this protocol expires 28 February 2007.** If any more changes are made to the study protocol you must notify the IRB and receive approval before implementation.

If you have questions, please contact the Institutional Review Board at OGRD (509) 335-9661. Any revised materials can be mailed to Research Compliance Office (Campus Zip 3140), faxed to (509) 335-1676, or in some cases by electronic mail, to [irb@wsu.edu](mailto:irb@wsu.edu).

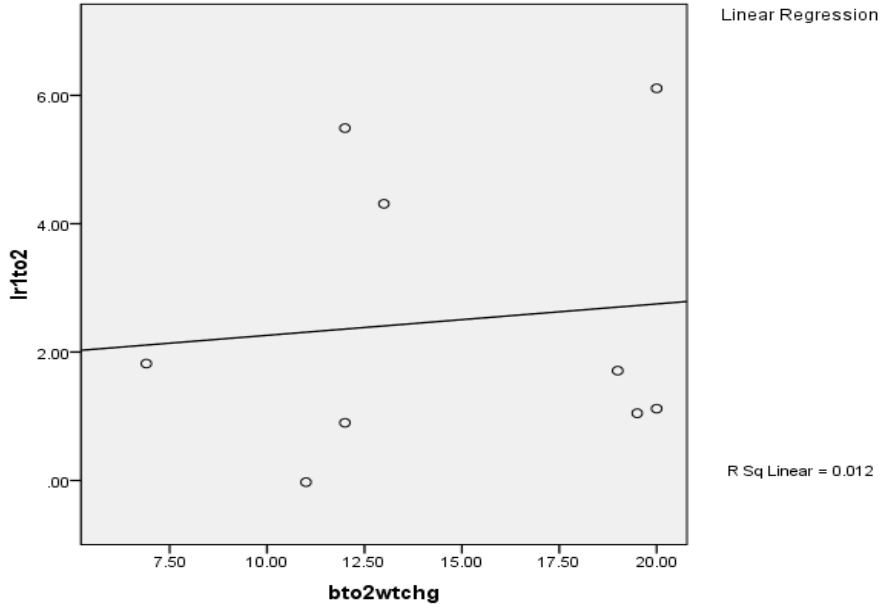
Review Type: MOD  
Review Category: EXP  
Date Received: 6 September 2006

OGRD No.: NF  
Agency: NA

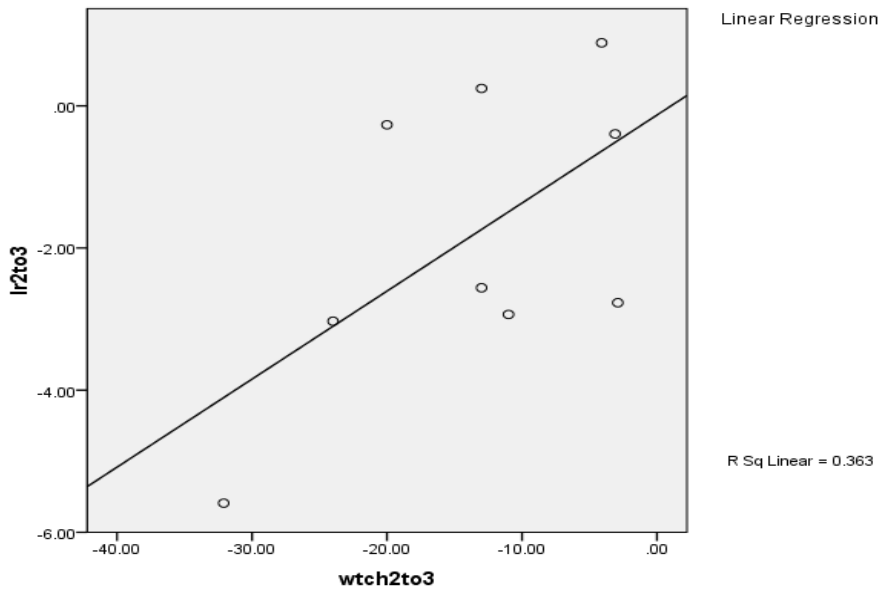
PO Box 643140, Pullman, WA 99164-3140 • 509-335-9661 • Fax: 509-335-1676  
[iacuc@wsu.edu](mailto:iacuc@wsu.edu) • [ibc@wsu.edu](mailto:ibc@wsu.edu) • [irb@wsu.edu](mailto:irb@wsu.edu) • [www.research-compliance.wsu.edu](http://www.research-compliance.wsu.edu)

## Appendix D

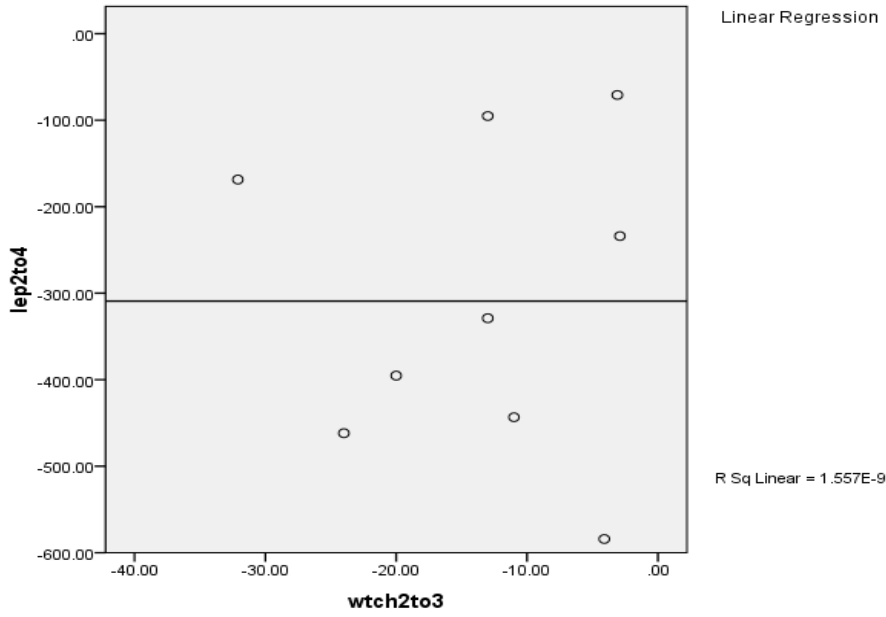
### Scatterplots of Variables



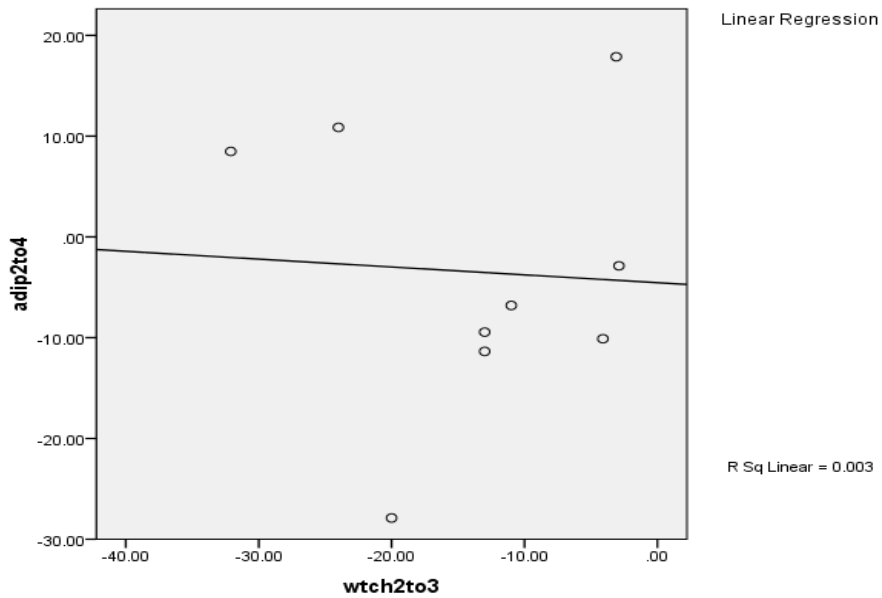
Scatterplot change comparison of HOMA IR T1 to T2 (Ir1to2) to Weight T1 to T2 (bto2wtchg)



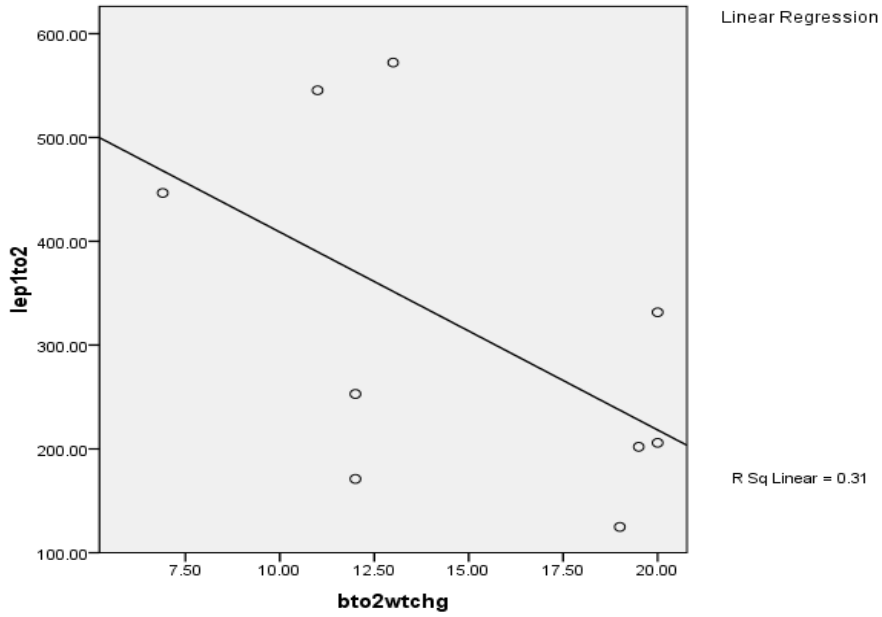
Scatterplot change comparison of HOMA IR T2 to T3 (Ir2to3) to weight T2 to T3 (wtch2to3).



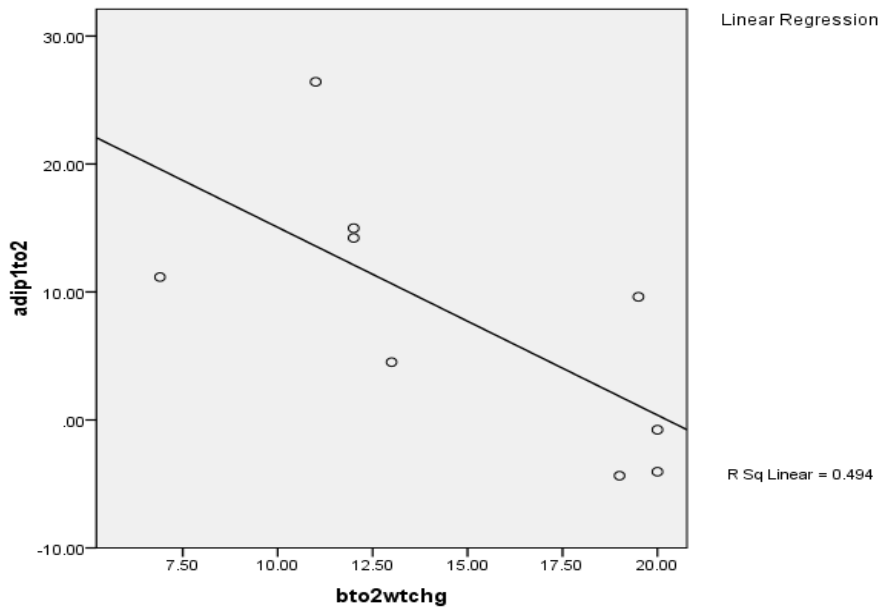
Scatterplot change comparison of Leptin T2 to T3 (lep2to4) to Weight T2 to T3 (wtch2to3).



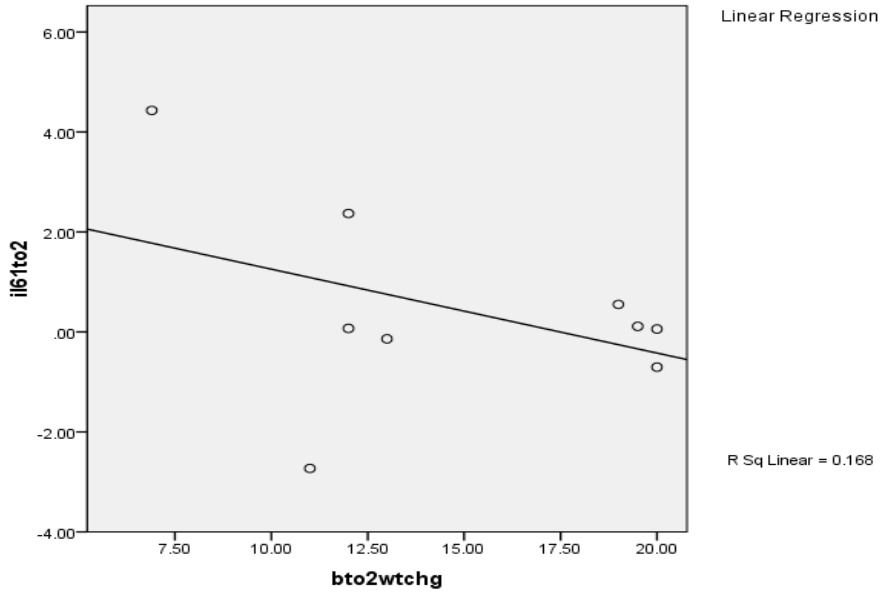
Scatterplot change comparison of Adiponectin T2 to T3 (adip2to4) and Weight T2 to T3 (wtch2to3).



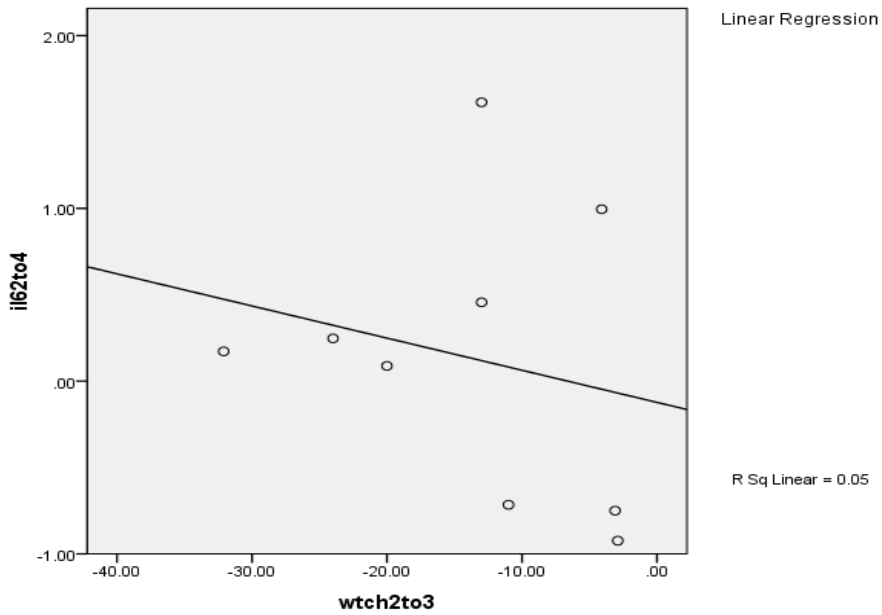
Scatterplot change comparison of Leptin T1 to T2 (lep1to2) and Weight T1 to T2 (bto2wtchg).



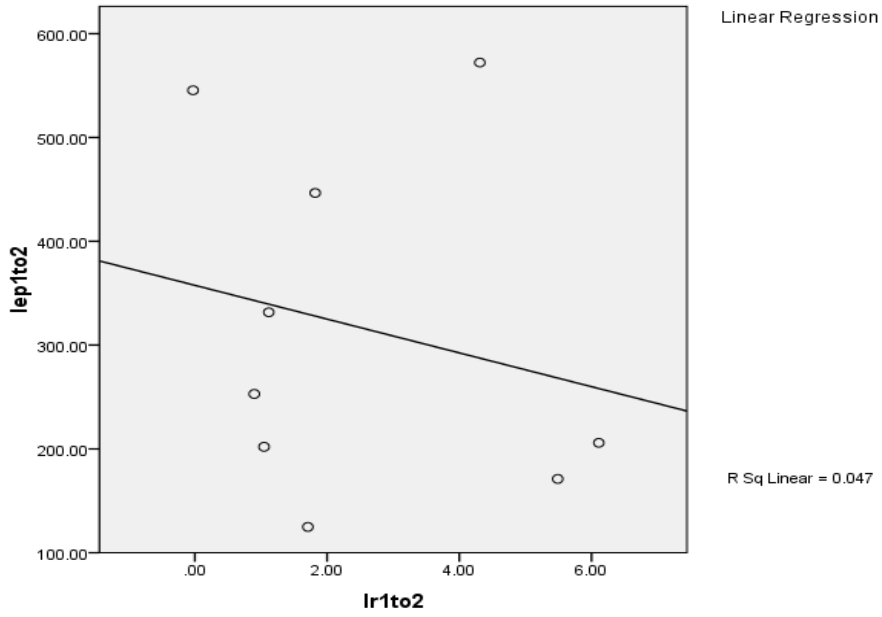
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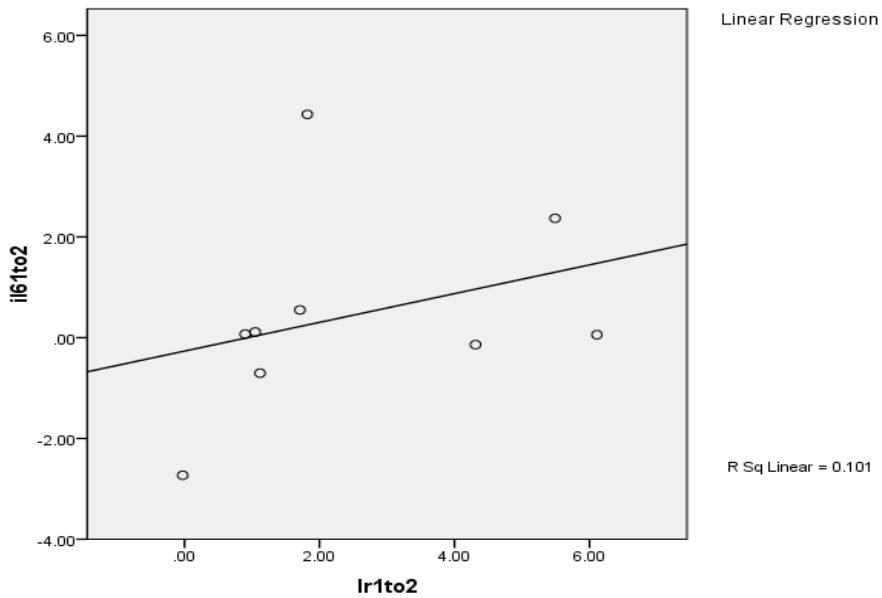
Scatterplot change comparisons of IL-6 T1 to T2 (il61to2) and Weight T1 to T2 (btowwtchg).



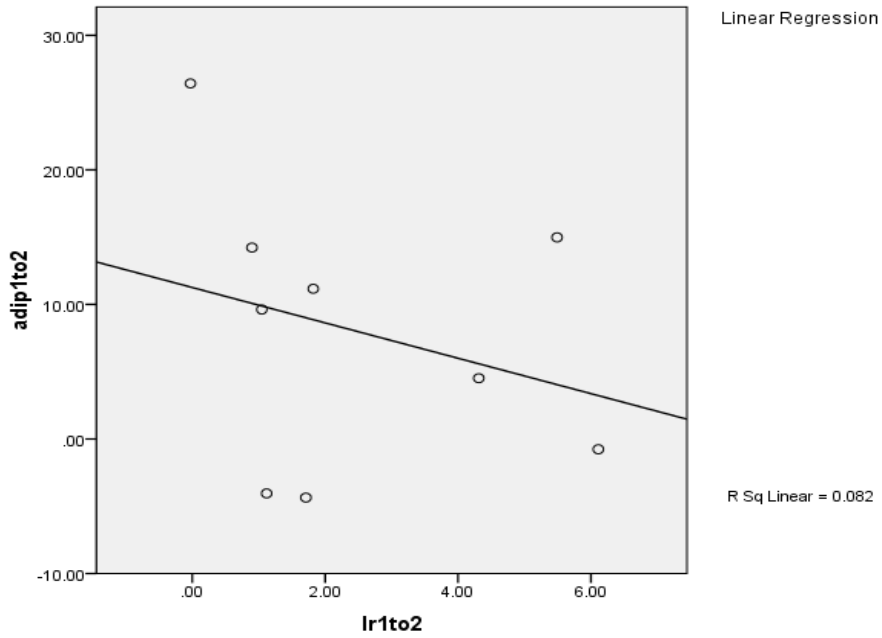
Scatterplot changes comparisons of IL-6 T2 to T3 (il62to4) and Weight T2 to T3 (wtch2to3).



Scatterplot change comparisons of Leptin T1 to T2 (lep1to2) and HOMA IR T1 to T2 (Ir1to2).



Scatterplot change comparisons of IL-6 T1 to T2 (il61to2) and HOMA IR T1 to T2 (Ir1to2).



Scatterplot change comparisons of Adiponectin T1 to T2 (adip1to2) and HOMA IR T1 to T2 (Ir1to2).