

Changes in Cardiotrophin-1 and Fibroblast Growth Factor-21 with Weight Loss

by

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Abstract

The purpose of this investigation was to examine the effects of modest weight loss on circulating concentrations of cardiostrophin-1 (CT-1) and fibroblast growth factor-21 (FGF-21) in obese men. Nine obese men (age = 41.5 ± 7.1 ; weight = 101.7 ± 21.0 kg; BMI = 32.8 ± 3.6 kg/m²; % fat = 35.2 ± 4.3) were assigned to either an exercise or dietary restriction intervention. Seven age-matched controls (age = 42.3 ± 8.5 ; weight = 74.5 ± 5.0 kg; BMI = 24.8 ± 1.4 kg/m²; % fat = 25.2 ± 5.5) served for comparison purposes. Control individuals were not assigned to an intervention. The overall targeted weight loss for both interventions was 8 to 10% of initial body weight over a 6 to 10 month period. A blood sample and DEXA scan were obtained for each participant at baseline and every four weeks during the intervention in the obese group. Blood samples were analyzed for CT-1, FGF-21, glucose, insulin, non-esterified fatty acids (NEFA), adiponectin, IL-6, TNF- α , myeloperoxidase (MPO), and total antioxidant capacity (TAC). Glucose to insulin ratio (GIR), homeostasis model index (HOMA) and the quantitative insulin sensitivity check index (QUICKI) were used as clinical indexes of metabolic health. Differences between groups were determined by independent t-test. Changes in blood variables were determined by repeated measures ANOVA and paired t-test. Pearson product moment correlation coefficients were used to determine the relationship between baseline physiological and metabolic characteristics and dependent variables ($p < 0.05$ for all). Neither CT-1 nor FGF-21 concentrations differed between groups at baseline. No significant change in CT-1 concentrations occurred with 8 to 10% weight loss. FGF-21 concentrations significantly decreased by 57.3% after weight loss of 8 to 10%. Significant reductions in total and regional body fat, lean mass, insulin, GIR, HOMA and QUICKI also occurred with weight loss. Reductions in FGF-21 occurred along with those in total and regional body fat and improvements in insulin sensitivity. These results indicate that FGF-21 may be a potential clinical indicator of improvements in metabolic regulation that occur with modest weight loss.

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Chapter I.

Introduction

Obesity and Metabolic Syndrome

Obesity is a major public health issue leading to a greater risk of all cause mortality and a predisposition to chronic diseases including atherosclerosis, hypertension, diabetes mellitus and cancer (37, 76, 126). Until recently the role of fat cells in the pathogenesis of obesity and metabolic syndrome was thought to be a passive one. Now adipose tissue is known to be an active endocrine organ, playing a major role in metabolic control (173, 177, 257, 440, 550).

Obesity develops due to an imbalance between energy intake and energy expenditure (341). Adipose tissue normally functions as the main storage area for dietary lipids. Increased dietary consumption without an increase in energy expenditure leads to adipose tissue growth and a reduction in adipose tissue lipid storage potential (173). Tissues that, under normal circumstances, do not function as lipid storage sites are forced to accommodate the excess energy causing ectopic fat accumulation at the liver, skeletal muscle, and pancreas (173, 177, 440). Consequently, increased hepatic glucose and very-low density lipoprotein production, elevated insulin secretion, as well as decreased skeletal muscle glucose uptake occur (173, 177). Excessive fatty acids in circulation and ectopic fat accumulation disrupt metabolic pathways and endocrine function in many tissues (257, 352). Moreover, the rapid accumulation of adipose tissue outpaces tissue circulation requirements, leading to a hypoxic state and eventual tissue necrosis (173). Macrophage infiltration and dysfunctional cytokine secretion take place in response to the hypoxic environment and perishing adipocytes, further leading to an insulin resistant state (173, 440). As such, obesity has been characterized by systemic oxidative stress caused by hyperglycemia, elevated tissue lipid levels, chronic inflammation, inadequate antioxidant defenses and reactive oxygen species (ROS) formation (571, 573).

Metabolic syndrome (MetS) is a constellation of interrelated conditions including central obesity, dyslipidemia, hypertension and hyperglycemia that directly promote cardiovascular disease (CVD), insulin resistance and type 2 diabetes (179, 182, 222). Obesity appears to contribute to MetS via abdominal body fat distribution as opposed to the absolute amount of adipose tissue (37). Therefore, excess intra-abdominal adiposity is key in the pathogenesis of obesity and a host of accompanying cardiovascular and metabolic disorders (35, 173, 177, 550).

Adipose tissue secretes a wide array of biologically active proteins called cytokines, or more specifically, adipocytokines or adipokines (37, 486). Since some of these factors greatly influence insulin sensitivity, glucose metabolism, inflammation and atherosclerosis, they may provide a molecular link between obesity, insulin resistance and the development of type 2 diabetes and cardiovascular disease (222). Some of the most well characterized adipocytokines are leptin, adiponectin, TNF- α and IL-6 (159, 173, 177, 222, 440, 550).

Adiponectin exerts insulin-sensitizing and anti-atherogenic effects that include lowering of hepatic glucose production and increasing glucose uptake and fatty acid oxidation in skeletal muscle (35, 222, 331). Adiponectin is down-regulated in obesity and a significant inverse relationship exists between adiponectin and markers of oxidative stress that are observed in states of excess obesity (159, 222, 331, 571, 573).

TNF- α plays a key role in mediating immune system response (372). TNF- α regulates the production of other cytokines such as IL-1 and IL-6, inflammation and cell apoptosis and promotes cell survival (372). TNF- α expression is increased in obesity and contributes to insulin resistance by directly inhibiting insulin-signaling (5, 550). Increased TNF- α levels induce the production of reactive oxygen species as part of its activity in the inflammatory response (634).

IL-6 is closely associated with obesity and insulin resistance (550). IL-6 stimulates an increased production of adhesion molecules in inflamed and dysfunctional tissue resulting in the recruitment of leukocytes to inflamed sites. IL-6 may also increase IL-1 and TNF- α synthesis (531). Elevated IL-6 expression leads to insulin resistance through a down-regulation of insulin receptor substrate (440, 457). Increased circulating concentrations of IL-6 are demonstrated with impaired glucose tolerance and

hyperlipidemia and are implicated in development of type 2 diabetes (31, 440, 575). Furthermore, reactive oxygen species raise the mRNA expression levels of IL-6 (159).

There are 50 to 100 adipocytokines that have been identified to date (486). The physiology of many of these proteins remains poorly understood and the functional roles are currently being characterized. Two novel bioactive peptides secreted from adipose tissue that have received considerable recent attention and may further explain the link between obesity, insulin resistance, cardiovascular disease and oxidative stress are cardiostrophin-1 and fibroblast growth factor-21 (73, 268, 308, 365, 636, 645).

Cardiostrophin-1 and Fibroblast Growth Factor-21

Cardiostrophin-1 (CT-1) was first identified in 1995 based on its ability to induce cardiac myocyte hypertrophy (422, 423). CT-1 expression is high in heart, skeletal muscle, prostate, ovaries, liver, lung, kidney and adipose tissues (365, 424). Expression of CT-1 is up-regulated in states of obesity and in individuals with MetS (334, 365). CT-1 has well documented effects on the myocardium that include hypertrophy and prevention of apoptosis in response to myocardial ischemia (299). Elevated CT-1 levels are found along with failing left ventricular myocardium, left ventricular hypertrophy, treated and untreated hypertension, and vascular dysfunction (319, 320, 334, 421, 453, 644). CT-1 contributes to the proposed etiology of MetS by inhibiting adipocyte insulin signaling and insulin-stimulated glucose uptake (525, 645). CT-1 and some ROS regulate one another through a positive feedback loop as CT-1 potentiates ROS production, which in turn, can lead to further CT-1 expression (21, 482). These data suggest that the close association between obesity, hypertension, insulin resistance, vascular inflammation and hypoxia may account for the high circulating concentrations of CT-1 observed with obesity and conditions contributing to MetS. Therefore, CT-1 concentrations may present a possible link between obesity, oxidative stress, cardiovascular disease and type 2 diabetes (365, 525).

In 2000, fibroblast growth factor-21 was first identified as a secreted protein preferentially expressed in the liver (374). Recently FGF-21 expression was found in adipose tissue (358, 636). It was not until 2005 that FGF-21 was determined to be a potent metabolic regulator (268). These metabolic actions include lowering blood

glucose through decreased insulin resistance and increased GLUT-1-mediated glucose uptake in adipocytes (268, 269, 355, 592). Reductions in fasting insulin, triglyceride and LDL-cholesterol levels are also reported with FGF-21 treatment (268, 269, 592). FGF-21 induces an increase in adipocyte PPAR- γ expression; thereby, increasing adiponectin expression and reducing oxidative stress by inhibiting the formation of reactive oxygen species (357, 358, 376, 389). FGF-21 administration in animals increased energy expenditure and fat oxidation, accelerated weight loss and decreased adiposity and body weight (89, 269). However, FGF-21 data in humans does not appear to be consistent with that seen in animal models. In humans, elevated levels of FGF-21 are observed in obesity, type 2 diabetes and MetS (73, 308, 636). FGF-21 concentrations are elevated along with BMI and body fat percentage as well (73, 111, 308, 636). Individuals with hypertension, elevated triglycerides, hyperglycemia and increased fasting insulin also exhibit increased FGF-21 concentrations (73, 307, 308, 636)

The elevated concentrations of FGF-21 in human obesity may be a result of FGF-21 resistance. However, other hypotheses for the greater FGF-21 concentrations in obese humans versus their normal weight counterparts have been proposed (475). Lower adiponectin levels observed with elevated FGF-21 concentrations in human obesity seem counter-intuitive (111, 636). Both adiponectin and FGF-21 are gene targets of the nuclear receptor PPAR- γ (357, 358, 580). PPAR- γ is suppressed in obesity and with oxidative stress (376). This relationship would predict decreased levels of FGF-21 in human obesity and states of elevated oxidative stress. The opposite has actually been observed suggesting a level of FGF-21 resistance (73, 308, 475, 636). At present, the manner in which excess adipose tissue and body weight and body fat loss influence concentrations of CT-1 and FGF-21 are not characterized. In addition, the metabolic effects obtained by changes in these adipocytokines with weight and body fat loss are not well understood.

Lifestyle Modification and Weight Loss

Weight loss through lifestyle modification (exercise and/or hypocaloric diet) is beneficial for treating excessive adiposity, dyslipidemia, hypertension, hyperglycemia and insulin resistance (87, 413). The magnitude of weight loss does not need to be large, as even modest weight loss of 5-10% significantly attenuates metabolic dysfunction (87). A decrease in caloric intake is an avenue by which to create a negative energy balance resulting in weight loss (476). It is prudent to recommend a reduced calorie diet low in saturated fat, higher in unsaturated fat, high in whole grains and low in sodium (87). However, more important than the composition of the diet, is the overall caloric intake. Regardless of which macronutrients are emphasized, hypo-caloric diets are key to inducing meaningful weight loss (476).

A lifestyle aimed at increasing physical activity/energy expenditure and decreasing, or even maintaining, body weight is another important approach to reducing health risks (87). Increased physical activity and higher cardio-respiratory fitness, independent of weight loss, are associated with decreased CVD risk and lower incidence of type 2 diabetes and MetS (87, 298, 383, 585, 622). Exercise is also particularly effective at improving insulin sensitivity as well as reducing dyslipidemia and hypertension (23, 87, 106, 474, 524). Cardiorespiratory fitness seems to have an independent effect on metabolic function in some aspects; however, a change in body weight and body composition (particularly abdominal adiposity), is an important mediator in the ability of increased physical activity to modify chronic disease risk (87). Regular exercise appears to play an important role in abdominal fat loss and the metabolic changes that ensue (87, 466).

The temporal effects of weight loss are not yet firmly established in the literature. However, evidence suggests that metabolic improvements may occur rapidly within the first few weeks of lifestyle modification and continue at a slower rate as weight loss continues. Clinical markers of metabolic health that demonstrate this trend include triglycerides, glucose, insulin, hemoglobin A1c, insulin sensitivity and biomarkers of oxidative stress (96, 262, 318, 345, 478, 513).

Purpose

Enlargement of adipocytes, impaired adipose tissue blood flow, adipose tissue hypoxia and macrophage infiltration of adipose tissue are interrelated and may lead to dysregulated adipokine secretion and ectopic fat storage, which together may result in insulin resistance and further chronic disease (173). The purpose of the present investigation is to examine the temporal effects of modest weight loss on circulating levels of CT-1 and FGF-21 in obese men. Both of these cytokines appear to affect insulin sensitivity and may possibly play a role in the link between obesity, insulin resistance and chronic disease. A secondary purpose is to characterize changes in CT-1 and FGF-21 with those observed for oxidative stress, cytokine responses and clinical markers of metabolic health.

Questions, Hypotheses and Rationale

Question:

1. What are the differences in circulating concentrations of CT-1 and FGF-21 between the control and obese individuals in our cohort?

Hypotheses:

Null: There are no differences in CT-1 and FGF-21 levels between obese middle-aged men and age matched controls.

Alternative: Circulating CT-1 and FGF-21 levels are significantly higher in obese individuals as compared to age-matched controls.

Rationale:

Obesity is characterized by excessive adipose tissue size, decreased adipose tissue storage capabilities, ectopic fat distribution, adipose tissue hypoxia, and dysregulated adipokine secretion (173, 177, 440). It has recently been determined that adipose tissue is a source of CT-1 and FGF-21 expression (365, 636). Furthermore, previous work demonstrates that there are differences in CT-1 and FGF-21 concentrations between

obese and lean individuals (73, 308, 365, 636). CT-1 expression is up-regulated in obese individuals and in persons with MetS (334, 365). Elevated levels of FGF-21 are also reported in states of obesity and in persons with type 2 diabetes and MetS (73, 308, 636). It appears that in obese individuals, levels of the cytokines CT-1 and FGF-21 will be elevated significantly versus concentrations observed in lean controls due to an increased amount of adipose tissue and ectopic fat accumulation contributing to vascular and tissue inflammation (37, 173, 177, 550).

Question:

2. What is the effect of 8-10% weight loss on circulating levels of CT-1 and FGF-21?

Hypotheses:

Null: Weight loss of 8-10% has no effect on circulating amounts of CT-1 and FGF-21.

Alternative: Weight loss of 8-10% significantly decreases circulating concentrations of CT-1 and FGF-21.

Rationale:

Weight loss through lifestyle modification is beneficial for treating excessive adiposity, dyslipidemia, hypertension, hyperglycemia and insulin resistance (87, 413). Modest weight reductions of 5-10% significantly attenuate metabolic dysfunction (87). Currently, there is no evidence in the literature that we know of regarding the effect of weight loss on CT-1 and FGF-21 concentrations. However, data show that adipose tissue is a source of CT-1 and FGF-21, which may account for the high circulating concentrations of these bioactive peptides observed in obese humans (73, 308, 365, 525, 636). Moreover, it is likely that vascular and tissue inflammation exists as a result of ectopic fat accumulation (37, 173, 177, 550). Since several tissues produce CT-1 and FGF-21, circulating levels may also be reflective of this vascular and non-adipose tissue inflammation (268, 365, 374, 424). As previously noted, weight loss, through diet or exercise, is effective in reducing body fat and excessive adiposity and in normalizing adipokine secretion (87, 413, 462). Thus, it appears prudent to predict that weight loss-

induced reductions in adipose tissue, ectopic fat accumulation and attenuation of dysregulated adipokine secretion will serve to lower circulating concentrations of CT-1 and FGF-21. Along with the possibility of increased adipose tissue expression of FGF-21, there are three rational explanations for why FGF-21 concentrations are increased in obesity. The increase may be an indirect sign of FGF-21 resistance, a protective mechanism or possibly indicates the presence of truncated, inactive form of the protein in circulation (475). If inactive isoforms are present in obesity, it may be that with weight loss FGF-21 levels do not decrease, but normal FGF-21 production and/or signaling is restored.

Question:

3. When do changes in circulating concentrations of CT-1 and FGF-21 occur and does the magnitude of change continue as weight reduction progresses?

Hypotheses:

Null: Progressive weight loss does not have an effect on circulating concentrations of CT-1 and FGF-21.

Alternative: Lifestyle intervention and weight loss initially causes a rapid change in circulating concentrations of CT-1 and FGF-21, which continues at a slower rate over time until the desired 8-10% weight loss is achieved.

Rationale:

In order to experience the health benefits associated with weight loss, the magnitude of weight loss does not need to be large, as even modest reductions of 5-10% significantly attenuate metabolic dysfunction (87). However, do these changes occur linearly over time or do they occur rapidly at first and then continue at a slower rate until the desired weight loss is achieved? Furthermore, are the changes related to total body fat or regional body fat reduction? In the literature, the answer to this question is not clear. A hypothesis regarding the temporal responses of FGF-21 and CT-1 to weight loss can be formulated through the responses of oxidative stress, glucose, insulin and insulin

sensitivity. Rapid reductions in markers of oxidative stress are seen before weight loss and changes in body composition occur with lifestyle modification (96, 318, 412, 478, 513, 571, 573). Reductions continue; however, at a slower rate throughout the intervention (96, 318, 345, 478, 513). In addition, total fat mass and abdominal visceral adipose tissue decrease in line with biomarkers of oxidative stress during weight loss (168).

FGF-21 is a gene target of PPAR- γ , which is up-regulated by weight loss and also known to reduce oxidative stress (357, 358, 376, 389, 568). Through the activity of PPAR- γ , oxidative stress and FGF-21 appear to be linked. This relationship suggests that FGF-21 levels will increase with reductions in oxidative stress occurring with weight loss. On the other hand, it is known that FGF-21 concentrations and oxidative stress levels are increased in obese humans (73, 308, 342, 571, 573, 636). A relevant hypothesis can be formulated for each case. However, based on human data it appears that FGF-21 concentrations will most likely decrease along with reductions in oxidative stress, increases in adiponectin and enhancements in insulin sensitivity during weight loss. Furthermore, if it is true that in obesity truncated, inactive forms of the protein are present, it could be postulated that FGF-21 levels will not decrease, but normal FGF-21 signaling/production will be restored with weight loss. CT-1 and reactive oxygen species directly regulate one another through a positive feedback loop as elevated concentrations of CT-1 induce production of ROS and vice versa (21, 482). Therefore, it appears that CT-1 levels will mirror reductions in markers of oxidative stress based on these data and data from cases of human obesity (21, 365, 482).

Reductions in glucose and insulin and enhancements in insulin sensitivity with weight loss appear to follow the same trend as do markers of oxidative stress (73, 87, 262, 292, 516). Based on interactions demonstrated in the literature, CT-1 and FGF-21 responses to weight loss may mirror those of insulin, glucose and insulin sensitivity (73, 111, 365, 636, 645). CT-1 expression is enhanced by glucose in a dose dependent manner and CT-1 levels are significantly higher in subjects with hyperglycemia (365). Furthermore, chronic administration of CT-1 causes insulin resistance and hyperinsulinemia (645). Also, CT-1 inhibits insulin action through decreasing the expression of IRS-1 (645). Fasting blood glucose and plasma insulin concentrations were

found to independently influence plasma FGF-21 levels (73). Increased FGF-21 concentrations were also reported along with elevated fasting insulin levels and decreased insulin sensitivity (73, 111, 636). These relationships with oxidative stress, glucose, insulin and insulin sensitivity provide evidence that the temporal changes in CT-1 and FGF-21 may occur rapidly at the outset of the weight loss intervention and then continue at a slower pace as weight loss progresses.

Question:

4. Do changes in circulating concentrations of CT-1 and FGF-21 occur along with improvements in clinical markers of metabolic health (glucose, insulin, NEFA, adiponectin, biomarkers of oxidative stress) known to occur with weight loss?

Hypotheses:

Null: Circulating amounts of CT-1 and FGF-21 are not associated with improvements in clinical markers of metabolic health known to occur with weight loss.

Alternative: Circulating concentrations of CT-1 and FGF-21 will decrease in conjunction with reductions in glucose, insulin, NEFAs and biomarkers of oxidative stress and increases in adiponectin levels.

Rationale:

Weight loss through lifestyle modification is effective in causing changes in clinical markers of metabolic health such as glucose, insulin, non-esterified fatty acids, adiponectin and reactive oxygen species (ROS) (87, 96, 142, 318, 373, 403, 412, 413, 439, 513, 568, 571). It is possible that alterations in circulating levels of CT-1 and FGF-21 may mirror these changes based on mechanisms and relationships presented in the literature. For example, CT-1 concentrations are elevated along with increased levels of insulin and glucose (365). CT-1 also down-regulates the nuclear receptor PPAR- γ most likely resulting in decreased expression of the PPAR- γ target and insulin sensitizing agent adiponectin (358, 645). Furthermore, CT-1 and reactive oxygen species appear to

regulate one another through a positive feedback loop in that CT-1 potentiates ROS production, which in turn, leads to further CT-1 expression (21, 482).

In humans, FGF-21 concentrations are elevated along with circulating fatty acids, glucose and insulin (73, 111, 308, 636). FGF-21 is also a gene target of PPAR- γ , which up-regulates adiponectin expression and inhibits the formation of ROS (357, 358, 580). Weight loss results in an up-regulation of PPAR- γ that should cause an increase in FGF-21 and adiponectin and a decrease in ROS (568). However, FGF-21 levels are already increased in states of human obesity hinting at possible FGF-21 resistance (73, 308, 475, 636). Based on these data, FGF-21 levels may decrease with weight loss, despite being a gene target of PPAR- γ . In other words, reductions in FGF-21 hypothesized here to occur with weight loss will likely occur along with elevations in adiponectin and decreases in biomarkers of oxidative stress. It is possible that the link between FGF-21 and levels of fatty acids, glucose and insulin may be stronger than the effects levied by weight loss-induced up-regulation of PPAR- γ . Based on these specific relationships, CT-1 and FGF-21 concentrations should decrease along with weight loss-induced reductions in glucose, insulin, NEFAs and biomarkers of oxidative stress and elevations in adiponectin demonstrated in the literature (87, 96, 142, 318, 373, 403, 412, 413, 439, 513, 568, 571).

Assumptions

1. A major contribution to changes in blood variables reflected metabolic shifts resulting from improvements in cardiovascular fitness and decreases in body weight, adipose tissue, hypoxia and inflammation.
2. The diet group remained physically inactive throughout the duration of the study.
3. The exercise group did not engage in a hypo-caloric diet during the study.
4. The exercise group exercised as instructed outside of the lab.
5. Both groups accurately reported their diet and physical activity.

Limitations

1. We could not control the participant's sleep habits, stress levels and outside physical activities.

2. There are inherent limitations to the diet recording process such as under estimating portion sizes and inaccurate reporting.
3. Nutrient composition and caloric intake were not controlled.
4. We were limited in the number of dependent blood variables that we could measure.

Delimitations

1. Only physically inactive males between the ages of 30 and 65 years were recruited for the study.
2. All participants had no prior history of extreme weight loss and had been relatively weight stable over the previous 6 months.
3. All participants were recruited from Auburn, Alabama and the surrounding areas.
4. An intensity of 60-70% of VO_2 max was used to expend the desired amount of calories for all exercise sessions.

Significance of Study

Excessive adiposity reflects a chronic imbalance of energy intake exceeding energy expenditure. In obese individuals, this excessive adiposity contributes to the development of metabolic dysfunction, in part, due to the inability to store additional lipids, ectopic fat distribution, the creation of a hypoxic state within the tissue, and abnormal adipokine secretion (173). On the other hand, weight loss through lifestyle modification is beneficial for treating excessive adiposity, dyslipidemia, hypertension, hyperglycemia and insulin resistance (87, 413). Weight loss generated by hypo-caloric diets and regularly practiced exercise can create the negative energy balance necessary to generate these beneficial effects. Recent evidence supports the theoretical role of cytokines and adipokines in obesity and weight loss (37, 126, 143, 222). Both CT-1 and FGF-21 may be related to the metabolic dysfunction occurring with obesity as well as play a role in the metabolic and cardiovascular health improvements associated with weight loss (268, 365, 636, 645). However, there appear to be limited data in the literature that directly addresses the response of CT-1 and FGF-21 to weight loss in humans. Therefore, the importance of this study lies in its potential to contribute

foundational information that will be useful and relevant to our understanding of the responses of CT-1 and FGF-21 to obesity and modest weight loss.

Chapter II.

Review of Literature

Obesity and Metabolic Syndrome

Overweight and obesity are a major public health issue affecting nearly half a billion of the world's population (469). The World Health Organization estimates that by 2015, the number of overweight people globally will increase to 2.3 billion and greater than 700 million will be obese (3). Once considered only a problem in high-income countries, obesity rates are now rising dramatically in developing nations (3). In the United States 30% of all adults are presently overweight and 32% are obese (178, 382). Obesity rates in Europe range from 10 to 20% in men and 10 to 25% in women (469). As a result, the cost of obesity is between 2 and 8% of total health care budgets in all parts of the world, irrespective of the health care system (469). In the United States alone, annual medical spending due to overweight and obesity is estimated as \$92.6 billion, which represents 9.1% of US health expenditures (135). Hospital costs and the use of medication increase with increasing BMI. In a large health maintenance organization, annual costs were 25% higher for individuals with a BMI between 30 and 35 kg/m² and 44% higher for a BMI greater than 35 kg/m² than in those with a BMI between 20 and 25 kg/m² (54, 443). In addition, complications associated with obesity contribute to 100,000 to 400,000 deaths per year (350). Data show that a BMI of greater than 30 reduces life expectancy by 3 to 5 years (418). Moreover, a 30% increase in all cause mortality was reported in persons with a BMI between the 85th and 95th percentiles (117). Both elevated healthcare costs and increased mortality are a result of the fact that obesity causes a predisposition to chronic diseases including atherosclerosis, hypertension, diabetes mellitus, cancer and sleep apnea (37, 76, 126, 434).

The two most common and alarming disorders resulting from obesity are type 2 diabetes and cardiovascular disease. Type 2 diabetes is tightly associated with obesity due to the combination of the modern diet and sedentary lifestyle (340). Adults with a BMI of 35 or more are roughly 20 times more likely to develop diabetes over a ten year period than their normal weight counterparts (134). As a result of skyrocketing obesity rates, the prevalence of diabetes is rising dramatically throughout the world. The number of adults with diabetes is predicted to increase by 46% from 151 million in 2000 to 221 million in 2010 (641). Furthermore, by 2025, the global number of individuals with type 2 diabetes could double from the number observed in 1995 (273).

Almost 81 million (1 in 3) American adults have 1 or more types of cardiovascular disease (CVD). Total CVD includes the disorders of hypertension, coronary heart disease (myocardial infarction and angina pectoris), heart failure and stroke. Mortality data show that CVD accounted for 36.3% of all deaths in 2004, or 1 of every 2.8 deaths in the United States. An average of 2,400 Americans die from CVD each day, an average of 1 death every 37 seconds (464). According to the NCHS, if all forms of major CVD were eliminated, life expectancy would rise by almost 7 years (10, 464). The estimated direct and indirect cost of CVD for 2008 was \$448.5 billion. Moreover, the total healthcare spending for CVD increased 8.06% from 1987 to 2000 (464, 548).

The relation between obesity and CVD is complicated. Some suggest that the connection is indirect and dependent on the increased prevalence of diabetes, hypertension and dyslipidemia, while others suggest an independent association between obesity and CVD risk (147, 180, 336). A recent meta-analysis of 57 studies including over 900,000 adults showed that for each 5 kg/m² increase above a BMI of 25 kg/m², cardiovascular mortality increases by 40% (597). However, the use of BMI as a CVD risk factor has been debated as BMI fails to consider body fat distribution and storage patterns (336). BMI is not a good index of visceral fat, which serves as the major fat depot contributing to increased metabolic and cardiovascular risk (145, 336). Waist-to-hip ratio (WHR) and waist circumference (WC) might be superior to BMI as risk assessment tools (336, 631). A recent meta-regression analysis concluded that a 1 cm increase in WC or a 0.01 increase in WHR elevated relative CVD risk by 2% and 5%

respectively (100). The INTERHEART study, on almost 30,000 acute myocardial infarction (AMI) patients from 52 countries, demonstrates a strong association between abdominal obesity and AMI risk worldwide. Furthermore, a WHR greater than 0.85 in women and greater than 0.90 in men is a risk factor for AMI (631).

The major pathophysiologic conditions associated with obesity are also involved in the pathogenesis of the metabolic syndrome (MetS) (87, 95, 181, 184). Metabolic syndrome is a constellation of interrelated conditions including abdominal obesity, dyslipidemia, hypertension and hyperglycemia that directly promote cardiovascular disease (CVD) and type 2 diabetes (179, 182, 222). Over the past decade a number of organizations have proposed different sets of criteria for defining MetS. The organizations include the World Health Organization (WHO), International Diabetes Federation, American Heart Association (AHA) and National Heart, Lung and Blood Institute (NHLBI). However, the most widely used diagnosis criteria are those from the National Cholesterol Education Program (NCEP) Adult Treatment III (ATP III) endorsed by the AHA and NHLBI (182). These criteria are based upon common clinical measures such as waist circumference, triglycerides, HDL-C, blood pressure, and fasting glucose level (see Table 1) (182, 184).

Table 1. NCEP ATP III: the metabolic syndrome criteria

Risk Factor	Defining Level
Adominal obesity (waist circumference)	
Men	> 102 cm (40 in)
Women	> 88 cm (35 in)
Triglycerides	> 150 mg/dL
HDL-C	
Men	< 40 mg/dL
Women	< 50 mg/dL
Blood pressure	
Systolic	> 130 mmHg
Diastolic	> 85 mmHg
Fasting glucose	> 100 mg/dL
*Diagnosis = when ≥ 3 of these risk factors are present	

The presence of defined abnormalities in any 3 of the 5 measures constitutes diagnosis of MetS (182).

Although the criteria differ slightly from one organization to the other, the respective definitions all serve the clinical function of identifying obese individuals who are at a greater risk of developing co-morbid metabolic conditions such as type 2 diabetes and cardiovascular disease (293, 603).

The prevalence of the metabolic syndrome is relatively high worldwide. The Third National Health and Nutrition Examination Survey (NHANES) reported that the overall prevalence of MetS in adults over the age of 20 years was 24% in the United States. This prevalence increased to greater than 30% in 50-year-old individuals and greater than 40% in individuals aged 60 years and over (184). When using the NCEP criteria, the prevalence of MetS is lower in Black Americans (140, 182). This is most likely due to lower average waist circumferences, lower triglycerides and higher HDL-C levels. On the other hand, Blacks are more susceptible to insulin resistance, hypertension and diabetes (178). Thus, the NCEP criteria may not provide a clear picture of the metabolic abnormalities occurring in this population (178). The greatest prevalence of MetS among any racial group in the United States was found in Hispanics, with 32% estimated to have MetS (139). The most likely reason for this is greater insulin resistance, glucose intolerance and hyperglycemia in this ethnic group (306). This is manifest in the fact that Hispanics have the highest rate of type 2 diabetes in the US (185). Furthermore, from a worldwide perspective, approximately one fourth of the European and Latin American populations are thought to exhibit MetS (178).

As previously noted, the risk for cardiovascular disease and type 2 diabetes rises dramatically in individuals with MetS (182, 293, 296). Prospective population studies show that MetS results in a ~ 2 fold increase in the relative risk for CVD as opposed to individuals without metabolic syndrome (182, 296). In the Framingham Heart Study population, MetS predicted roughly 25% of all new-onset CVD (600). The ten-year risk for developing CVD in men with MetS was in the range of 10-20% (181). Furthermore, Lakka and colleagues (296) reported that men with metabolic syndrome, as defined by NCEP ATP III, were 2.9 to 4.2 times more likely to die of CHD. MetS as defined by the WHO was associated with 2.6 to 3.0 times higher CVD mortality (296). Prospective population studies also show a ~ 5 fold increase in risk for developing type 2 diabetes as compared to people without the syndrome (182). The presence of METs, in both men and women in the Framingham cohort, was highly predictive of new-onset diabetes (182). Moreover, almost half of the attributable risk for type 2 diabetes could be

explained by the presence of MetS as defined by NCEP ATP III standards (181). As reported by Laaksonen and colleagues (293), men who met the WHO definition of MetS in which adiposity was defined as waist-to-hip-ratio > 0.90 or body mass index ≥ 30 kg/m² had a ~ 9 fold greater risk of developing diabetes than men without the syndrome. Men fulfilling the WHO definition of MetS where adiposity was defined as ≥ 94 cm were 7 times more likely to develop diabetes (293). When using the NCEP ATP III criteria with abdominal adiposity defined as ≥ 102 cm, the risk for developing diabetes was 5 times greater than that for men without MetS (293)

Pathophysiology of Obesity

Although the relationship between obesity, insulin resistance and cardiovascular disease is well-recognized, the mechanisms involved remain relatively poorly understood (173, 181). It is evident that adipocytes are very important for health as shown using a lipotrophic mouse model. These mice have almost no white fat tissue and had characteristics similar to those seen in humans with lipotrophic diabetes, insulin resistance, hyperglycemia, hyperlipidemia, and fatty livers (164, 507). Transplantation of adipose tissue from healthy mice resulted in attenuated hyperglycemia, accompanied by lowered insulin concentrations, improved muscle insulin sensitivity, decreased serum triglycerides, decreased hepatic gluconeogenesis and decreased amounts of fat deposited in the muscle and liver (164). Thus, fat cells obviously play a role in health and the absence of adipocytes is “metabolically detrimental” (177).

Adipose tissue dysfunction appears to contribute to obesity related insulin resistance and type 2 diabetes (485, 508). Evidence suggests that enlarged adipocytes, impaired adipose tissue blood flow, adipose tissue hypoxia, local inflammation in adipose tissue and adipose tissue macrophage infiltration seem to be interrelated and may lead to disturbances in adipokine secretion, lipid overflow, and ectopic fat storage, which together may result in the development and progression of insulin resistance and type 2 diabetes (173).

Adipose Tissue Size

The enlargement of adipocytes is frequently observed in obesity and is proposed to be involved in the pathogenesis of insulin resistance and type 2 diabetes (118, 173, 407, 593). Increased adipocyte size may be the result of impaired differentiation, which appears to be a precipitating factor in the development of type 2 diabetes and an independent marker of insulin resistance (326, 595). A possible link between adipocyte size and insulin resistance may be the release of fatty acids from adipose tissue (173). The mobilization of fatty acids from stored adipocyte triglycerides is mediated by hormone sensitive lipase and adipose triglyceride lipase and is strongly inhibited by insulin (207, 640). Some have suggested that adipocytes of obese individuals have become resistant to the anti-lipolytic effects of insulin, thus resulting in the increased release of fatty acids from the tissue into circulation (173). However, non-esterified fatty acid release into circulation may not be the only link between adipocyte size and insulin resistance as diabetic subjects are equally responsive to the anti-lipolytic effects of insulin compared to control subjects despite the presence of systemic insulin resistance (17, 317, 326, 593).

Since obese adipose tissue may be normally responsive to the anti-lipolytic effects of insulin, the hyperinsulinemia often present in insulin resistant conditions might decrease fasting lipolysis; thereby, protecting obese individuals from the deleterious effects of high circulating fatty acid concentrations (256). On the other hand, decreased lipolysis may also contribute to a continuous increase in adipocyte size, which may further impair the adipose tissue dynamic storage capabilities (173). Impaired adipose tissue lipid buffering capacity, rather than increased fasting adipocyte lipolysis, may play a greater role in the prolonged elevation of circulating non-esterified fatty acids, thus serving as the link between adipocyte size and insulin resistance (86).

Furthermore, studies report that the insulin-sensitizing agents TZDs, acting via activation of PPAR γ , stimulate adipocyte differentiation resulting in an increase in the number of small adipocytes with large capacitance (102, 187, 388). In addition, TZDs promote the trafficking of bone marrow-derived circulating progenitor cells to adipose tissue and stimulate their differentiation into adipocytes (92). A novel TZD has been shown to induce adipocyte differentiation in Zucker diabetic fatty rats by stimulating

PPAR γ , thus increasing the number of small adipocytes and improving insulin sensitivity (347). In summary, one theory as to how adipocyte size is linked to insulin resistance is based on the ability of enlarged adipocytes to effectively store dietary fatty acids. Consequently, lipid overflow, ectopic fat distribution and insulin resistance may develop (173).

Adipose Tissue Storage Capability and Ectopic Fat Deposition

Adipose tissue is the main lipid storage depot. In obese individuals, whose energy intake exceeds energy expenditure, adipose tissue is overloaded with triglycerides and the additional lipid storage capability is diminished. In other words, adipose tissue is a “metabolic sink” that can reach capacity. Triglyceride storage in the adipocytes of obese subjects has reached a near maximum levels, thus these adipocytes cannot effectively store excess lipids. Consequently, non-adipose tissues such as skeletal muscle, the liver and the pancreas are exposed to free fatty acids that remain in circulation leading to lipid storage redistribution in these tissues (146, 173).

There is a plethora of information suggesting that ectopic fat distribution in non-adipose tissue plays a crucial role in the development of insulin resistance and impaired insulin secretion (28, 41, 141, 234, 280, 404). Animal studies have shown a direct relationship between the accumulation of fatty acid derived metabolites in the liver or skeletal muscle and insulin resistance mediated by alterations in the insulin-signaling pathway (271). In the liver and skeletal muscle, imaging studies have illustrated an inverse linear relationship between insulin-mediated glucose disposal and the presence of intra-myocellular and hepatic fat (340). Similarly Greco and colleagues (176) demonstrated that lipid deprivation in skeletal muscle selectively depletes inter- and intra-myocellular lipid stores and reverses insulin resistance. Work from a number of laboratories has shown that in both rodents and humans, the triglyceride content of muscle bears a negative relationship to whole-body insulin sensitivity (404, 488). Increased delivery of fatty acids to the liver leads to increased hepatic glucose production, elevated hepatic very low-density lipoprotein and triglyceride output, and reduced insulin clearance by the liver, resulting in conditions associated with insulin resistance, such as glucose intolerance, hyperlipidemia, and hyperinsulinemia (63, 125,

133, 149, 201, 305, 534, 598). Moreover, there is evidence that chronic exposure of pancreatic β -cells to elevated free fatty acid (FFA) levels can be damaging to their function (44, 479, 504, 638). Isolated islets exposed to high concentrations of FFA for periods of 24-48 hours typically show enhanced insulin secretion at low glucose concentrations, depressed proinsulin biosynthesis, depletion of insulin stores, and an impaired response of the β -cell to stimulatory concentrations of glucose (44, 638). Similar findings were also found in intact rats and Zucker diabetic fatty rats (479, 504).

The mechanism by which elevated free fatty acids in circulation and ectopic fat deposition result in insulin resistance is not fully understood. However, it is possible that fatty acids and potentially several metabolites including acyl-CoAs, ceramides, and diacylglycerol serve as signaling molecules that activate protein kinases such as protein kinase C (PKC), Jun kinase (JNK), and the inhibitor of nuclear factor- κ B (NF- κ B) kinase- β (IKK β). These kinases can subsequently impair insulin signaling by stimulating the inhibitory serine phosphorylation of insulin receptor substrates (IRS), the key mediators of insulin receptor signaling (430, 440). Based on these data, it appears that an impaired storage potential of dietary fat in adipose tissue may result in ectopic fat deposition and insulin resistance in situations where energy intake exceeds energy expenditure (173).

Adipose Tissue Blood Flow

Blood flow may be an important regulator of metabolism in adipose tissue (60, 160, 480). It is possible that disturbances in normal adipose tissue blood flow might contribute to an increased lipid supply to non-adipose tissues resulting in ectopic fat deposition and insulin resistance (173). In lean healthy individuals, adipose tissue blood flow is responsive to nutrient intake, which may be important in the regulation of metabolism by facilitating signaling between adipose tissue and other tissues (12, 13, 86, 123, 148, 175). Blood flow to the adipose tissue controls the interaction between circulating triglyceride rich lipoprotein particles and lipoprotein lipase, which hydrolyzes these particles into fatty acids and glycerol (480). Evidence suggests that both fasting and postprandial adipose tissue blood flow is diminished in obese states and may contribute to the reduced fasting and postprandial triglyceride extraction/clearance in

adipose tissue in obese compared to lean individuals (42, 174, 239, 436, 533, 574). Moreover, intravenous adrenaline infusion causing an elevation of adipose tissue blood flow resulted in the increased clearance of circulating triglycerides into adipose tissue (480). Therefore, decreased adipose tissue blood flow may play a role in decreased triglyceride extraction, lipid overflow, ectopic fat distribution and lipid-induced insulin resistance (173).

Adipose Tissue Hypoxia

Adipose tissue hypoxia, which is closely related to adipose tissue blood flow, may contribute to obesity related impairments in adipokine expression/secretion and subsequent insulin resistance (173). Adipocyte size increases up to 140-180 μm in diameter during the development of obesity (57). These enlarged adipocytes encounter less than adequate oxygen supply since the maximum diffusion distance for oxygen is 100 μm (200). Hosogai et al. (212) demonstrated that white adipose tissue is hypoxic and exhibits markedly increased lactate concentration in obese versus lean mice. Furthermore, weight loss appears to decrease the expression of hypoxia responsive genes (66).

A likely explanation for adipose tissue hypoxia is based on blood flow through the tissue (173). Trayhurn and colleagues (555) have recently proposed that the expansion of adipose tissue mass during the progression of obesity may lead to hypoxia in parts of the tissue because angiogenesis is insufficient to maintain normoxia in the entire adipose tissue depot. This may lead to increased production of inflammatory factors, acute phase proteins and angiogenic factors by adipose tissue (173). These events most likely involve the key controller of the cellular response to hypoxia, the transcription factor hypoxia-inducible factor-1 (HIF-1) (554-556). Therefore, decreased adipose tissue blood flow and the resultant hypoxia in obesity appear to be integral to the development of adipocyte dysfunction and the subsequent metabolic and cardiovascular complications described previously.

Hypoxic cells respond by altering gene expression in an attempt to ensure adaptation (173). Hypoxia causes the expression of HIF-1 α , which combines with HIF-1 β to form the transcription factor HIF-1 (211, 495, 591). This transcription factor

regulates the response to alterations in oxygen supply and the expression of genes that are involved in angiogenesis, erythropoiesis, inflammation and glucose metabolism (211, 316, 496, 591). Hypoxia may lead to disturbances in adipose tissue glucose homeostasis through inducing changes in the expression of glucose transporters (607). Furthermore, recent data suggest that hypoxia dysregulates the expression of several adipokines known to affect glucose homeostasis and insulin sensitivity. For example, adiponectin and PPAR γ mRNA expression were reduced while PAI-1 and visfatin mRNA expression were elevated in hypoxic 3T3-L1 adipocytes when compared to normoxic cells (212, 493). Similarly, hypoxia induces PAI-1 production and inhibits adiponectin synthesis in 3T3-L1 adipocytes (71). Adipose tissue hypoxia in obese mice was also associated with elevations in inflammatory genes and decreased expression of adiponectin (623). Recent data show that hypoxia induces HIF-1 α protein synthesis, while increasing the expression of inflammatory adipokines in human adipocytes (579). HIF-1 α expression is also related to macrophage infiltration of obese adipose tissue (66). When macrophages experience hypoxic conditions, they signal for further macrophage recruitment and the activation of other inflammatory cells (304).

Furthermore, cell death may occur in response to hypoxia. The severity of hypoxia determines whether apoptosis occurs or if adipocytes adapt and survive (173). Adipose tissue macrophages are reportedly predominantly localized to necrotic adipocytes (79). Adipocyte death was positively associated with adipocyte size in obese mice and humans and in hormone-sensitive lipase deficient mice (79). Thus, adipocyte death may induce excess macrophage recruitment to adipose tissue of obese individuals (173). Hypoxia also inhibits adipocyte differentiation, which may be regulated by the production of mitochondrial reactive oxygen species (68, 69, 630). As mentioned previously, impaired adipocyte differentiation appears to be causally related to the development of type 2 diabetes (407, 593).

Based on these presented data, adipose tissue hypoxia, most likely due to inadequate adipose tissue blood flow in response to increased adipose tissue size, may play an important role in the link between obesity and insulin resistance through effects on macrophage infiltration, adipokine expression and/or adipocyte differentiation.

Adipose Tissue Inflammation, Macrophage Infiltration and Dysregulated Adipokine Secretion

Obesity is associated with a state of chronic, low-grade inflammation characterized by abnormal cytokine production and the activation of inflammatory signaling pathways in adipose tissue (213, 222). Obese hypertrophic adipocytes and stromal cells within the white adipose tissue directly augment systemic inflammation (222). White adipose tissue typically consists of a 5-10% macrophage population, however, a rodent model of diet-induced obesity causes a significant increase in macrophage infiltration, with macrophages consisting of 60% of all cells found in white adipose tissue (587). Macrophages are now recognized as important non-adipocyte cells that contribute to the production of adipose tissue inflammatory factors (173). It has been reported that non-adipose cells in adipose tissue are responsible for the majority of secreted inflammatory factors, except for leptin and adiponectin, which are primarily produced by adipocytes (124). Adipose tissue macrophages are responsible for nearly all TNF- α expression and a significant portion of IL-6 expression (101, 587). Enhancements in inflammatory cytokine expression by white adipose tissue are associated with a parallel increase in white adipose tissue macrophage content (66, 587, 610). Thus, it appears that several adipokines implicated in systemic inflammation are cytokines produced by macrophages in the adipose tissue.

Obese adipose tissue is characterized by progressive infiltration by macrophages as obesity develops (173, 587, 589, 610). Macrophage infiltration in adipose tissue is positively associated with body mass index, adipocyte size and insulin resistance (66, 94, 108, 587, 610). Moreover, weight loss results in a regression of adipocyte hypertrophy and macrophage infiltration and an improvement in the inflammatory profile of gene expression (66, 80). It has been reported that macrophage-secreted factors impair human fat cell differentiation and induce inflammatory events in 3T3-L1 adipocytes by activating the NF- κ B pathway (294, 427). Thus, it seems that macrophage infiltration may play an important role in the inflammatory response of obese adipose tissue (173).

Initial studies focusing on the role of macrophages in insulin signaling illustrated that inhibition of the macrophage inflammatory pathway I κ B protects mice from obesity-induced insulin resistance (15). Arkan and colleagues(15) found that liver-

specific IkkB deletion in mice resulted in protection from high fat diet-induced hepatic insulin resistance. Moreover, these mice demonstrated a significant reduction in the expression of inflammatory markers in the liver (15). This study also showed that tissue-specific deletion of myeloid cell IkkB led to enhanced systemic insulin sensitivity with improved insulin action in skeletal muscle, liver and fat (15). Therefore, inactivation of the inflammatory pathway IkkB seems to prevent local and systemic insulin resistance, most likely by interrupting the local paracrine effects between resident macrophages and insulin target tissues (15). Furthermore, JNK-1 is an important component of obesity-induced insulin resistance (101). Solinas et al. (514) show that JNK-1 signaling in macrophages is a key component of macrophage function and a mediator of the macrophage inflammatory response, which subsequently leads to insulin resistance.

It has recently been determined that monocyte chemoattractant protein-1 (MCP-1), a chemokine and member of the small inducible cytokine family, may play an important role in macrophage infiltration of adipose tissue (173). Macrophages, endothelial cells and adipocytes produce MCP-1 and its expression is closely related to the number of resident macrophages (58, 75, 165). MCP-1 expression is increased in obese rodents and concentrations are elevated in obese and diabetic humans (75, 270, 375, 431, 481, 536). Furthermore, concentrations of MCP-1 have been found to decrease with weight-loss (487). Elevated MCP-1 expression is found in the early stages of obesity, suggesting that MCP-1 is initially produced by cells other than recruited macrophages (610). In addition, MCP-1 expression/secretion can be induced by TNF- α , IL-6 and insulin in pre-adipocytes and 3T3-L1 adipocytes, while its secretion can be suppressed by the PPAR- γ agonists, TZD's, and adiponectin (110, 127, 432, 481, 494, 610). Therefore, dysregulation of adipokine secretion in obesity may alter MCP-1 expression/secretion, which in turn can influence the extent of macrophage infiltration in adipose tissue (173).

MCP-1 knockout mice are characterized by decreased adipose tissue macrophage infiltration, reduced pro-inflammatory gene expression in adipose tissue, decreased hepatic triglyceride content and improved insulin sensitivity as compared to wild type animals (250, 586). On the other hand, adipose tissue-specific overexpression of MCP-1 increases adipose tissue macrophage infiltration and decreases insulin sensitivity (248,

250). It seems that MCP-1 is important in the process of macrophage infiltration in obese adipose tissue and may contribute to the development of obesity-related insulin resistance.

It appears to be improbable that macrophages initiate inflammation and dysregulated adipokine secretion in obese adipose tissue. Macrophages most likely augment the inflammatory signals that have already been established (173). Whatever the initial stimulus to recruit macrophages into adipose tissue is, once these cells are present and active, they, along with adipocytes and other cell types, could perpetuate a cycle of macrophage recruitment, production of inflammatory cytokines, and impairment of adipocyte function (589). The exact mechanisms underlying the disturbances in adipokine secretion and macrophage infiltration in obesity are yet to be fully elucidated. However, it is highly likely that these events relate to disruption within the adipose tissue itself such as the previously discussed increase in adipose tissue size, decrease in adipose tissue blood flow and adipose tissue hypoxia (173).

Oxidative Stress

Oxidative stress is an imbalance between reactive oxygen species (ROS), tissue free radicals and antioxidants that may possibly be a mechanism underlying obesity related disorders (571). Low levels of free radicals and ROS are necessary for normal cell redox state, cell function and intracellular signaling (370). High concentrations of free radicals and ROS damage DNA, proteins, carbohydrates and lipid constituents and compromise cell function (573, 626). Furthermore, ROS might be a key feature in the pathogenesis of the obesity associated metabolic disease, as recent evidence suggests that increased oxidative stress in accumulated fat is an early instigator of insulin resistance (159, 209, 218). Oxidative stress correlates with fat accumulation in humans and mice and may be a causative factor in the development of insulin resistance (122, 188, 440, 465). This is supported by studies showing that a reversal of the imbalance between ROS and antioxidants improves insulin resistance in humans and rodents (159, 183, 264, 330).

Possible mechanisms that generate oxidative stress/ROS in obesity include hyperglycemia, tissue lipids, inadequate antioxidant defenses, chronic inflammation, and hyperleptinemia. Several oxidative pathways are activated by hyperglycemia(573).

Advanced glycosylation end products (AGE) formed from proteins, lipids and nucleic acids are diabetic precursors. AGEs bind to specific cell surface receptors and lead to post-receptor signaling and generation of ROS (573). AGEs also activate intracellular transcription factors such as NF- κ B, which activates protein kinase C and sorbitol and transcription of vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1). Activation of these cellular molecules can produce ROS, as shown in rodent vessel tissues (460). As a result, oxidative damage and accelerated monocyte binding to the endothelium occur (19, 122). Hyperglycemia also stimulates the polyol pathway through which aldose reductase mediates the conversion of glucose to sorbitol (122). Elevated sorbitol levels in animal models cause oxidative damage and activate stress genes (122). Moreover, hyperglycemia induces NADPH oxidase activity, which serves to produce the ROS, superoxide, especially in the endothelium (635). Finally, recent data suggest that diet-induced increases in adipocyte glucose uptake in obese mice result in elevated ROS production (540).

Oxidative stress may partly be the result of the metabolic impact of intracellular triglycerides, which are elevated in obesity. Excessive triglycerides, by suppressing mitochondrial adenine nucleotide transporters, may increase ROS production within the mitochondrial electron transport chain (25). This decreases intramitochondrial adenine diphosphate causing electrons to build up within the electron transport chain, allowing them to react with oxygen to form superoxide (25). Visceral adiposity is also linked to elevated circulating FFA concentrations. FFA can acutely increase ROS formation in culture (224).

Lipids, such as LDL-C, serve as a major substrate for oxidation, stimulate radical formation, and enhance the accumulation of oxidative by products, especially in white adipose tissue (159). Excess lipid oxidation enhances the risk for thrombosis, endothelial dysfunction, and atherosclerosis (327, 461). Elevated lipid concentrations present in obesity may simply serve as an enlarged target for oxidative modification by ROS (391, 572). Circulating concentrations of biomarkers of oxidative stress were significantly elevated in obese vs lean Zucker rats. The major contributor to lipid peroxidation in the high fat fed Zucker rat was tissue lipid content (572). Furukawa and colleagues (159) discovered that in several obese mice models (KKAy, *db/db*, diet-induced, C57BL/6)

accumulation of fat in white adipocytes increased oxidative stress in white adipose tissue. Moreover, the KKAY mouse exhibited increased NADPH oxidase activity (generating ROS) and reduced activity and mRNA of the antioxidant enzymes superoxide dismutase, glutathione peroxidase and catalase in white adipose tissue (159). Based on these data, it appears that obesity induces ROS formation and lowers antioxidant defenses, thus promoting oxidative damage in the adipose tissue (573). Furukawa and colleagues (159) further explain that ROS and biomarkers of lipid peroxidation enter into circulation and initiate a vicious cycle of systemic oxidative stress in obesity.

Many human studies based on the link between oxidative stress and insulin resistance focus on the generation of ROS by hyperglycemia in diabetic patients. This suggests ROS as a consequence of diabetes-induced hyperglycemia and not a causative factor for insulin resistance (121, 122, 440). However, insulin resistance first presents itself long before the development of chronic fasting hyperglycemia, thus it is unlikely that insulin resistance in the pre-diabetic stage results from oxidative stress triggered by hyperglycemia (103, 440, 451). The increase in ROS in the pre-diabetic stage is more likely a result of obesity-induced elevations in FFAs that cause increased mitochondrial uncoupling (see following section) and β oxidation, leading to increased production of ROS and oxidative stress (67, 448, 604, 614). In healthy individuals, infusion of FFAs causes increased oxidative stress and insulin resistance that is reversed by infusion of antioxidants (405, 406).

Adequate tissue, dietary, enzymatic and non-enzymatic antioxidant defenses are critical to maintain antioxidant-prooxidant balance in tissues (573). Disturbances in this balance occur in obesity. Inadequate antioxidant defenses in obesity may begin with a low intake of antioxidant and phytochemical rich foods such as fruits, vegetables, whole grains, legumes, etc. Phytochemical intake is inversely correlated with waist circumference, BMI, and plasma lipid peroxidation (240). Moreover, dietary antioxidant levels are inversely related to the degree of adiposity (455, 577). Plasma vitamin concentrations also progressively decline as BMI increases (354, 532). In adults, blood retinol, tocopherol, vitamin C and carotene concentrations were 18-37% lower in obese women as compared to lean counterparts (354). Similarly, zinc levels were 38% lower in obese versus lean men (399). These data suggest that elevated BMI and obesity are

related to an increasing imbalance in the antioxidant-prooxidant status (573). In addition, dietary antioxidants may be used more rapidly in combating excessive prooxidant processes in obese individuals, leaving these subjects susceptible to free radical damage (573).

Activities of the major antioxidant enzymes may also be decreased in obesity (573). Erythrocyte superoxide dismutase and glutathione peroxidase activities were 29-42% lower in obese, high fat fed rats as opposed to control animals (33). On the other hand, in the early stages of obesity there may actually be increased activity of antioxidant enzymes in order to combat oxidative stress. However, as obesity progresses the sources of antioxidant enzymes are depleted (391, 572). Moreover, the degree of adiposity seems to affect enzyme activities. Olusi and colleagues (391) found that erythrocyte superoxide dismutase activity and glutathione peroxidase activity were lower in obese individuals versus non-obese controls. Furthermore, Ozata et al. (399) found 75 and 42% lower erythrocyte glutathione peroxidase and superoxide dismutase activities respectively in obese men compared to non-obese men. The combination of inadequate dietary, blood and enzymatic antioxidants and increased production of ROS creates an imbalance that favors oxidative stress in obesity (573).

Obesity in humans is characterized by a chronic state of low-grade inflammation (279, 596). Inflammation is defined by increased inflammatory cytokine expression and increased white blood cell counts and white cell activity (573). Fat is an active endocrine organ that expresses pro-inflammatory cytokines such as TNF- α and IL-6. The expansion of adipose tissue in obesity is associated with increased concentrations of TNF- α and IL-6, which may be released by adipocytes themselves or resident macrophages, as discussed previously (279, 328). Reduction of fat mass through weight loss is directly related to reductions in inflammatory cytokines in both humans and animals (97, 327). The cytokine profile may shift toward a prooxidant state in obesity and toward a balanced prooxidant-antioxidant state with optimal weight (573).

White blood cell counts are higher in obese individuals with elevations occurring in the monocyte and neutrophil subfractions (282). Monocytes produce ROS and oxidative enzymes, and when developed into macrophages they produce TNF- α and IL-6

(573). Neutrophils generate superoxide via NADPH oxidase (163). Both monocytes and neutrophils can convert hydrogen peroxide into ROS via myeloperoxidase (163).

As explained earlier, there is a progressive infiltration of macrophages into adipose tissue as obesity develops (173). This is in part due to the chemoattraction of leukocytes into adipose tissue by MDA and 4HNE (by-products of fat induced ROS generation) (159). It has been proposed that in obesity adipocyte-initiated macrophage recruitment and cytokine/ROS production by these macrophages occurs, which could potentially lead to oxidative damage and disease processes such as atherosclerosis (573, 589).

In summary, obese individuals display increased inflammatory cytokine levels, indirectly causing ROS formation via several intracellular signaling pathways (NF- κ B, NADPH), hyperglycemia and insulin receptor impairment. Leukocyte infiltration causes the enzymatic formation of ROS via NADPH oxidase. Both pathways (cytokines, leukocytes) generate ROS and induce oxidative stress (573).

Plasma leptin concentrations are proportional to the amount of adipose tissue and have a potential role in obesity-induced oxidative stress (84, 573). Leptin can directly stimulate production of ROS in cultured endothelial cells (49). Wistar rats injected with leptin displayed elevated plasma levels of various biomarkers of oxidative stress as compared to non-treated controls (34). Moreover, leptin indirectly stimulates production of inflammatory cytokines such as IL-6 and TNF- α (377). These cytokines increase NADPH oxidase production, which in turn generates ROS (377). Finally, leptin reduces the activity of the cellular antioxidant, paraoxonase-1 (PON-1). This reduction is related to increased levels of various biomarkers of oxidative stress (34). Therefore, hyperleptinemia may be involved in several mechanisms that promote oxidative stress in obesity (573).

Recent investigations have explored the molecular mechanisms through which oxidative stress might lead to insulin resistance (440). ROS and oxidative stress lead to the activation of several serine/threonine kinase signaling cascades in vitro (121, 291). These activated kinases act on a number of potential targets including the insulin receptor and the family of IRS proteins. An increase in serine phosphorylation on IRS-1 and IRS-2 inhibits the insulin signal that normally induces activating tyrosine phosphorylation (40,

121). The kinases found to be activated by oxidative stress include JNK, p38 MAPK, and IKK β (5, 43, 206, 218, 330, 627). Specific attention has been paid to the activity of JNK. ROS-induced insulin resistance may be mediated by JNK as increased ROS levels are known to stimulate threonine phosphorylation of JNK (206, 218, 252). This postulation is supported by evidence showing that the inhibition of JNK activity, through genetic knockout or an inhibitory peptide, improves insulin sensitivity in mice (206, 247, 251, 252).

Other Mechanisms Involved in the Pathophysiology of Obesity

Toll-like receptor 4 (TLR-4) is stimulated by lipopolysaccharide, an endotoxin released by gram-negative bacteria (101). TLR-4 stimulation results in the activation of IkkB/NF κ B and JNK/AP-1 signaling, up-regulating the expression and secretion of proinflammatory cytokines in adipose tissue and resident macrophages, including, IL-6, TNF- α and MCP-1 among others (231). TLR-4 was first shown to be a component in fatty acid-induced inflammation in studies examining the ability of dietary fatty acids to activate the inflammatory response via TLR-4 signaling in cultured macrophages (303). Shi and colleagues (499) discovered that free fatty acids (FFA) activate the NF κ B signaling pathway in primary macrophages from wild type mice, but not in macrophages harvested from TLR-4 deficient mice. Furthermore, *in vivo*, obese mice have increased TLR-4 expression in adipose tissue compared to lean controls (499). TLR-4 deficient mice are protected from lipid infusion-induced insulin resistance, due to decreased FFA-induced NF κ B transcriptional activity, as well as reduced expression of TNF- α , IL-6 and MCP-1 in adipose tissue (499). The work of Shi et al. (499) implicates TLR-4 in the development of lipid and obesity-induced insulin resistance and suggests that TLR-4 can act as a potential intracellular sensor of elevated tissue or serum FFA concentrations that are commonly found in obese individuals. Furthermore, recent data suggests that TLR-4 specifically located in macrophages and hepatic Kupffer cells participates in a sensing mechanism facilitating fatty acid-induced inflammation and insulin resistance (104, 109).

Another potential mechanism of inflammation and insulin resistance in obesity is endoplasmic reticulum (ER) stress (101, 173, 440). This is based upon the idea that overnutrition causes mechanical stress, excess lipid accumulation and protein synthesis,

and abnormal energy metabolism, all of which lead to an overburdened ER (101). It has been reported that ER stress is elevated in the adipose tissue and liver of obese mice (364, 401). This ER stress disrupts the normal folding of proteins and activates the unfolded protein response (UPR), an HIF-1-independent signaling pathway (39). Many disturbances, including hypoxia, cause accumulation of unfolded proteins in the ER, leading to ER stress (39, 129). Ozcan et al. (401) demonstrated that obesity imposes a strain on the ER machinery, triggering an ER stress response that activates JNK-mediated serine phosphorylation of IRS-1, resulting in an inhibition of the insulin-signaling pathway.

Newly synthesized proteins are regularly folded and assembled by chaperones in the ER (260). Recent studies have shown protection against obesity-induced type 2 diabetes in mice through overexpression of ER chaperones, while a knockdown of these chaperones resulted in an increased incidence of T2D (364, 400). Similarly, obese mice deficient for one allele of X-box binding protein-1, a transcription factor that promotes expression of molecular chaperones in response to ER stress, are more severely insulin resistant compared to obese controls (401). Oral administration of active chemical chaperones reduces ER stress and improves glucose homeostasis in obese mice (402).

The mechanism that leads to ER stress is not fully understood (440). Ectopic lipid deposition may trigger ER stress by sheer mechanical stress, perturbations in intracellular nutrient and energy fluxes or severe changes in tissue architecture (401). In addition, chronic increases in FFA concentration may induce ER stress (254). Finally, ER stress might lead to an increase in oxidative stress that in turn may contribute to insulin resistance (193).

Insulin resistance and type 2 diabetes mellitus are associated with a decrease in mitochondrial function that contributes to ectopic fat accumulation (430, 440). Petersen and colleagues (429) discovered that in elderly subjects severe insulin resistance is associated with significantly higher levels of triglycerides in both muscle and liver. This was accompanied by decreases in mitochondrial oxidative activity and mitochondrial ATP synthesis (429). Studies further suggest that insulin resistant individuals accumulate intramyocellular fat due to a decrease in the number of muscle mitochondria caused by reductions in the expression of nuclear-encoded genes that regulate mitochondrial

biogenesis, such as PGC-1 α and PGC-1 β (520, 609). Microarray studies show that PGC-1-responsive genes are down-regulated in obese Caucasians and Mexican-Americans with impaired glucose tolerance and type 2 diabetes (356, 415). Finally, activation of PGC-1 α is associated with improved mitochondrial function and insulin sensitivity in both animals and humans (295, 343). Therefore, insulin resistance might arise from defects in mitochondrial function, which in turn lead to increases in intracellular fatty acid metabolites (diacylglycerol, fatty acyl-CoA, ceramides) that disrupt insulin signaling (440).

A decrease in mitochondrial function in obese and insulin resistant individuals may seem counter-intuitive given that it is known that functional mitochondria are needed for the fatty acid-induced increase in ROS (122). It may be possible that the increase in ROS from fatty acid oxidation occurs early in the development of insulin resistance before mitochondrial dysfunction occurs (440). In the later stages of insulin resistance, ROS might promote a decrease in mitochondrial function that then leads to ectopic fat deposition, thus exacerbating the insulin resistant phenotype in obese individuals (440).

Some endocrinologists have proposed that obesity results from an increased endogenous production of the glucocorticoid hormone cortisol (177). Endogenous production as well as exogenous administration of cortisol results in weight gain and an increase in visceral fat deposition (177). Furthermore, elevated glucocorticoid levels cause insulin resistance and type 2 diabetes, primarily by opposing the anti-gluconeogenic effects of insulin in the liver (492). Hypercortisolemia, additionally, results in hyperphagia, central obesity and high concentrations of VLDL (177).

Circulating cortisol levels are near normal in obese individuals (192). However, adipose tissue does contain 11 β HSD-1, which converts the inactive metabolite, cortisone, to cortisol (440). Concentrations and activity of 11 β HSD-1 are elevated in adipose tissue of obese humans and rodents (313, 416, 449, 576). Transgenic overexpression, to about the same extent as is seen in obese humans, of 11 β HSD-1 selectively in mouse adipose tissue results in visceral obesity, insulin resistance and diabetes, increased cytokine expression, hyperphagia, hyperlipidemia and hypertension (338). Moreover, liver-specific antagonism of glucocorticoid action reduces hepatic glucose output and improves

glucose homeostasis in animal models of obesity-induced insulin resistance (235). Therefore, increases in endogenous 11 β HSD-1 appear to contribute to obesity-associated insulin resistance, possibly due to the delivery of cortisol/glucocorticoids to the liver via the portal vein (440).

Cytokines, Cardiotrophin 1 and Fibroblast Growth Factor-21

Adipose tissue secretes a wide array of biologically active proteins called cytokines, or more specifically, adipocytokines or adipokines (37, 486). Cytokines are a diverse group of proteins produced by a variety of cells that exert their influences through autocrine, paracrine and endocrine manners (45, 128). Stanley Cohen (82) first introduced the term “cytokine” in 1974. Until then the term “lymphokine” was used to describe proteins secreted from a variety of cell sources, affecting the growth and function of many types of cells (112, 547). In 1989, Balkwill and Burke (27) further defined cytokines as a group of protein regulators, which are produced by a wide variety of cells in the body, play an important role in many physiological responses, are involved in the pathophysiology of a range of diseases, and have therapeutic potential.

Currently, over 50 cytokines have been identified and characterized (486, 547). Cytokines are classified into several groups: interleukins, tumor necrosis factors, interferons, colony stimulating factors, transforming growth factors, and chemokines (547). These bioactive proteins are involved in intercellular communication, which regulates fundamental biological processes such as body growth, lactation, adiposity and hematopoiesis (48). They are especially important in regulating inflammatory and immune responses and have crucial functions in controlling both innate and adaptive immunity. The actions of cytokines are redundant, both functionally and temporally as they share a number of specific functions and features. The fact that cytokines have a local mode of action sets them apart from classical hormones (547). Cytokines show a wide variety of activities and can trigger several different cellular responses depending on cell type, timing and context. Also, they act synergistically in that the association of two cytokines amplifies their activity. Cytokines may also function in a juxtacrine manner stimulating cells that produce them, adjacent cells and cells throughout the body, or intervene in direct cell-to-cell interaction (128, 547). Lastly, these bioactive peptides

commonly share cytokine receptor subunits (547). For example, the IL-6 family of cytokines shares the gp130 subunit (359). Based on this redundancy and these shared functions and features, no single cytokine alone is likely to be solely responsible for controlling a specific cellular function or physiologic process (128).

A bulk of the current literature focuses on the roles of cytokines in the pathogenesis of disease. However, we know this group of proteins is involved in regulating normal physiologic responses. Even though elevated or suppressed cytokine production may be related to diseased states, cytokines are also involved in normal regulation of physiologic processes (128). With this being said, abnormalities in cytokines, their receptors and the signaling pathways that they initiate are involved in a wide variety of diseases (130). The cytokines, and possibly more appropriately adipokines or adipocytokines, that may provide a molecular link between obesity and the development of MetS, type 2 diabetes and cardiovascular disease are of particular interest. Some of the better-characterized cytokines involved with adipose tissue dysfunction include adiponectin, leptin, TNF- α , and IL-6.

General Cytokine Signaling Pathways (see Figure 2)

1. IKK β /NF- κ B

Cytokine-mediated inflammatory signals can impair insulin receptor and insulin receptor substrate (IRS) signaling through multiple signal transduction cascades (37, 603). Until recently the exact intracellular mechanism by which this occurred was not clear. Experiments using in vitro models of insulin resistance have shown that serine/threonine kinases phosphorylate insulin receptor and IRS molecules such that the necessary activation of the insulin signal by tyrosine phosphorylation is prevented (441). Considerable attention has now focused on the IKK β complex as a mediator of insulin resistance (603). IKK β can impact insulin signaling by directly phosphorylating inhibitory serine residues on IRS-1 and by phosphorylating inhibitor of NF- κ B (I κ B), thus activating the NF- κ B pathway, a crucial second messenger system for inflammatory cytokine signaling (440, 509, 603). More specifically, when stimulated, cytokine receptors recruit kinases that activate the IKK complex. IKK2, a catalytic subunit within the IKK complex, then phosphorylates I κ B thus allowing the transcription factor NF- κ B

to translocate into the nucleus and activate inflammatory target genes (255). This might trigger inflammatory responses that further dysregulate proper insulin signaling (440). Two groups have recently shown the relationship between IKK β expression and insulin resistance. Cai et al. (64) created a state of chronic low-grade inflammation in the liver in a transgenic mouse model via selective activation of NF- κ B. This caused continuous expression of IKK β . These mice exhibited a type 2 diabetic phenotype with evidence of moderate systemic insulin resistance (64). Similarly, Arkan and colleagues (15) used mice lacking IKK β in hepatocytes or myeloid cells. The deletion in hepatocytes resulted in increased insulin sensitivity in the mice when placed on a high fat diet or intercrossed with the *ob/ob* model of genetic obesity. Mice deficient in myeloid IKK β also demonstrated enhanced insulin sensitivity (15).

Through this pathway pro-inflammatory cytokines such as TNF- α stimulate the transcription of cytokines and adhesion molecules in peripheral tissues. These adhesion molecules, in turn, are elevated along with plasma lipids in atherosclerosis and diabetes, suggesting a role for NF- κ B in the pathogenesis of these chronic diseases (437, 535). In addition, activated NF- κ B has been found in smooth muscle cells and macrophages in human atherosclerotic lesions (46, 50, 351). NF- κ B activation in these cells controls the expression of the pro-inflammatory cytokines TNF- α , IL-6 and IL-8, as shown by their selective inhibition following NF- κ B blockade (351). Adiponectin suppresses TNF- α induced NF- κ B signaling at a step just before IKK β activation, thereby protecting against the development of long term comorbidities such as CVD and type 2 diabetes (397).

NF- κ B is a redox sensitive transcription factor, as the intracellular redox status of the cell is extremely important in the regulation of NF- κ B activity (238). Cytokine-stimulated activation of NF- κ B increases production of NO, which serves as a substrate for the formation of reactive oxygen species (ROS) (83). As described previously, ROS contribute to obesity related oxidative stress and likely mediate the pathogenesis of insulin resistance and atherosclerosis (22, 83, 122, 310, 515). Antioxidants, such as aspirin, NAC and flavanoids can inhibit cytokine-induced activation of NF- κ B and subsequent formation of ROS (546) (see figure 2).

2. *JNK*

c-Jun N-terminal kinase (JNK) has recently emerged as an important regulator of insulin resistance in obesity (206, 550). The JNK group controls cellular functions through control of the transcription factor activator protein-1 (AP-1) (550). A number of proinflammatory cytokines such as TNF- α , IL-6, IL-2 and MCP-1 are regulated by the JNK pathway through the interaction of AP-1 and sequences in their promoters (369).

JNK phosphorylation is mediated by two MAPK kinases, MAP2K4 and MAP2K7, which serve to cooperatively activate JNK. Gene disruption studies in mice show that both of these MAPKs are necessary for full activation of JNK and that MKK7 is essential for JNK activation by proinflammatory cytokines (553).

In both genetic and dietary animal models of obesity, JNK1 activity is increased in the liver, skeletal muscle and adipose tissue (206). Specifically, JNK1 is thought to likely mediate the crosstalk between inflammatory and metabolic signaling through activation by inflammatory stimuli. Moreover, the loss of JNK1 prevents the development of insulin resistance in these models (206). Liver specific knockout of JNK1 lowers circulating glucose and insulin concentrations, further solidifying its role in the development of insulin resistance (620) (see figure 2).

3. *JAK/STAT*

A number of cytokines also activate JAK and/or STAT proteins. Cytokines induce activation of their cognate receptors, resulting in activation of associated JAK kinases (JAK1, JAK2, JAK3) (378). Activated JAKs phosphorylate receptor cytoplasmic domains. Among the phosphorylated substrates are members of the STAT family of proteins (378). Receptor engagement and tyrosine phosphorylation activate the cytosolic STATs, resulting in nuclear translocation and gene activation. In particular, IL-6 binds to its receptor and activates JAK1 and STAT3 (547). STAT3 can be activated by a number of cytokines, especially those of the IL-6 family, mediating the expression of several acute phase response genes. STAT3 appears to play a critical negative role in controlling inflammation as shown in mice with STAT3 deletion (253, 538, 539, 590). See figure 1 for an overview of JAK/STAT signaling (230).

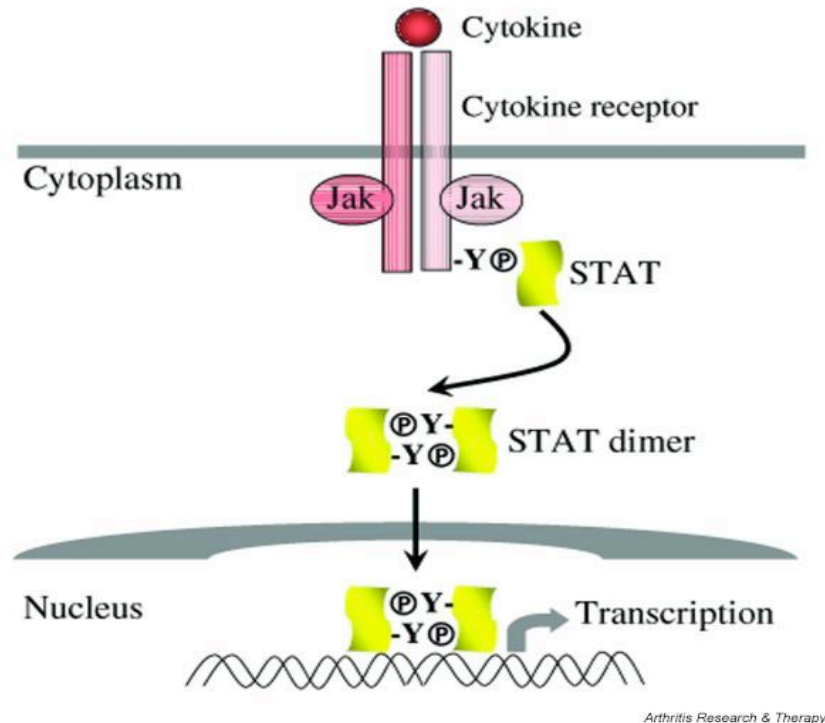


Figure 1. JAK/STAT signaling. The cytokine binds to the receptor resulting in activation of Jak-kinases and phosphorylation of receptor tyrosine residues. STATs dock on the phosphorylated tyrosine residues. STATs are then phosphorylated and translocate to the nucleus to activate gene transcription.

Cytokine signaling by the JAK/STAT pathway is regulated, in part, by a family of endogenous JAK kinase inhibitor proteins called suppressors of cytokine signaling (SOCS) (608). These inflammatory mediators constitute a negative feedback pathway in cytokine signaling and contribute to obesity-induced insulin resistance (440). At least three members of the SOCS family (SOCS-1, SOCS-3, SOCS-6) have been implicated in cytokine-mediated inhibition of insulin signaling (116, 353, 472). This occurs either by interference with IRS-1 and IRS-2 tyrosine phosphorylation, or by targeting IRS-1 and IRS-2 for proteosomal degradation (472, 564). Recent studies report that SOCS-3 levels were elevated in obese rodents and reductions in SOCS-3 expression resulted in resistance to high-fat-diet-induced obesity and insulin resistance (219, 500, 564). Furthermore, overexpression of SOCS-1 and SOCS-3 in the liver caused systemic insulin resistance (565) (see figure 2).

4. PPAR- γ

The peroxisomal proliferating-activated receptors (PPARs) are lipid sensing transcription factors that are primarily known to modulate energy metabolism, lipid storage/transport, inflammation and wound healing, as well as reducing oxidative stress (376). PPAR- γ is a member of the PPAR family that is most abundantly expressed in adipose tissue and heterodimerizes with retinoid X receptor (RXR). The heterodimer of RXR and PPAR- γ binds to a specific DNA sequence of PPAR responsive elements (PPRE) to activate several genes, especially the group involved in adipocyte differentiation and lipid metabolism (77, 228). Furthermore, PPAR- γ is intimately involved in the regulation of lipid and glucose homeostasis and insulin sensitivity as mutations in PPAR- γ result in insulin resistance, hypertension and diabetes (30). PPAR- γ is the molecular target of TZDs, pharmacological agents that exert insulin-sensitizing effects in adipose tissue, skeletal muscle and liver and negatively regulate the production of various pro-inflammatory cytokines that promote insulin resistance (550) (see figure 2).

In macrophages, that prominently invade adipose tissue in states of obesity as discussed earlier, PPAR- γ expression inhibits toll like receptor and IFN- γ mediated inflammatory responses (550). Resident macrophages in adipose tissue of lean mice display the alternatively activated or M2 phenotype characterized by activated genes for such anti-inflammatory cytokines as IL-10 (323). However, classic M1 pro-inflammatory macrophages are recruited to sites of tissue damage in the adipose tissue as in obesity. M1 macrophages produce enhanced levels of pro-inflammatory cytokines such as TNF- α (550). Obesity forces the state of adipose tissue macrophages from an M2 state that protects adipocytes from inflammation, to an M1 pro-inflammatory state leading to insulin resistance (550). Recent evidence suggests that this phenotypic alteration in adipose tissue macrophage polarization is governed by PPAR- γ , as PPAR- γ is required for the maturation of M2 macrophages (380). Based on these discussed data, the transcription factor PPAR- γ appears to play a role in cytokine production and signaling.

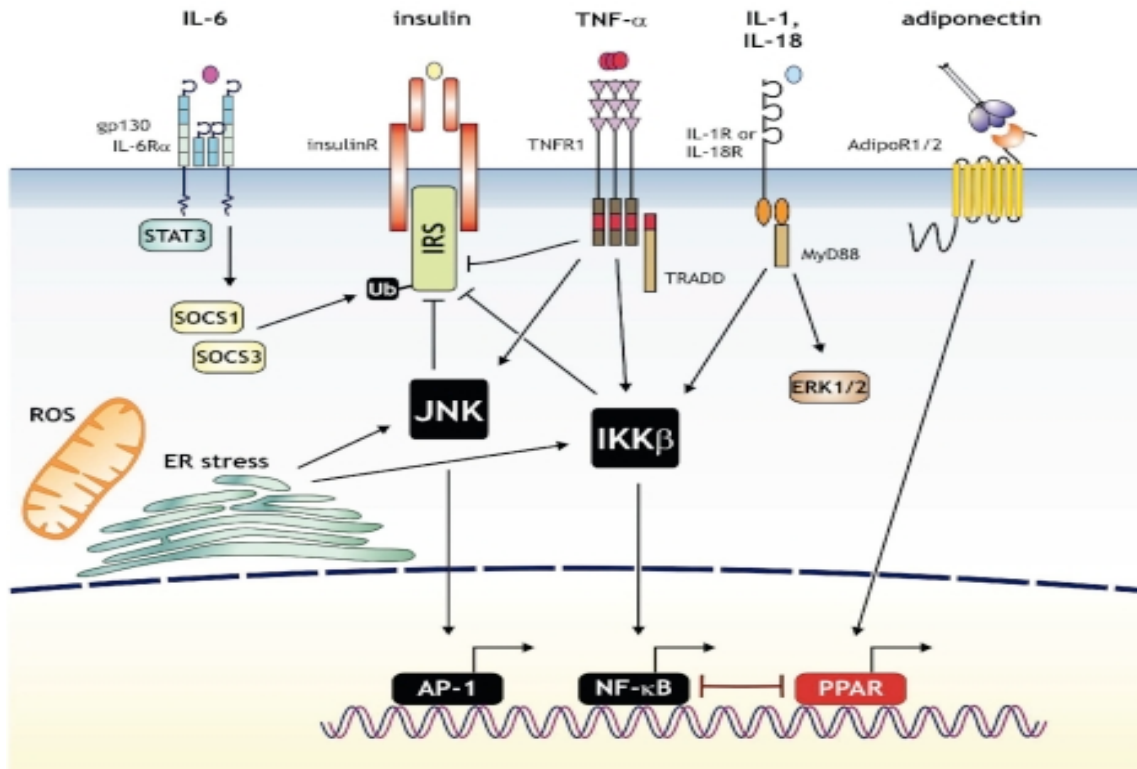


Figure 2. Overview of cytokine signaling. The engagement of TNFR by TNF- α activates IKK β /NF- κ B and JNK pathways, two major intracellular regulators of insulin resistance. Adiponectin signals via adiporeceptors to enhance PPAR activity. IL-1 and IL-18 can reduce IRS-1 expression via ERK 1/2 and can activate the IKK β /NF- κ B pathway. IL-6 can induce SOCS1 and SOCS3 to inhibit IRS-1. ER stress and oxidative stress are both involved in the activation of JNK and IKK β (550).

Cardiotrophin-1

Cardiotrophin-1 (CT-1), a 201 amino acid protein, was first identified in 1995 through expression cloning based on its ability to induce cardiac myocyte hypertrophy in vitro (422, 423). Amino acid sequence similarity shows that cardiotrophin-1 is a member of the interleukin-6 cytokine family (423). This family of cytokines has a wide range of growth and differentiation activities on many cell types including those from the blood, liver and nervous system (7, 274, 423). Cardiotrophin-1 is expressed in the adult human heart, skeletal muscle, ovary, colon, prostate and adipose tissue as well as fetal kidney and lung (365, 422, 424). The expression pattern of CT-1 and its range of activities in hematopoietic, neuronal, and developmental assays suggest that CT-1 may play an important role in other organ systems, in addition to its actions in cardiac development

and hypertrophy (425, 525). See tables 2 and 3 for factors regulating CT-1 expression and regulated by CT-1 (525).

Table 2. Factors regulating CT-1 expression

Angiotensin II	Increases CT-1 in cardiac fibroblasts. CT-1 increases angiotensinogen mRNA expression in cardiac myocytes. Up-regulation of angiotensinogen and angiotensin II production contribute to CT-1-induced cardiac myocyte hypertrophy (157).
Norepinephrine	Increases the expression of CT-1 mRNA in cardiac myocytes in vivo and in vitro (158).
Urocortin	Increases expression of CT-1 protein and mRNA (237).
FGF-2	Increases CT-1 in cardiomyocytes (241).
Glucose and Insulin	Increase CT-1 expression in human and murine adipocytes and neonatal rat cardiomyocytes (312, 365)
Reactive Oxygen Species	Increase CT-1 expression in murine embryonic stem cells (21, 482).

Table 3. Factors regulated by CT-1

HSP	CT-1 increases synthesis of hsp70 and hsp90. CT-1 increases hsp56 protein and mRNA (166, 236, 445).
PPAR- γ	CT-1 causes a transient decrease in PPAR- γ mRNA in 3T3-L1 adipocytes (645).
IL-6	CT-1 increases IL-6 mRNA and protein (154, 459).
STAT-1, -3, -5A, -5B, ERK1 and -2	CT-1 increases activation and nuclear translocation of STAT-1, -3, -5A, -5B, ERK-1 and -2 (482, 645).
MCP-1	CT-1 increases MCP-1 mRNA; STAT-3 phosphorylation, activation of JAK-2 and NF- κ B are involved in this mechanism (153).
Fatty acid synthase and IRS-1	CT-1 decreases fatty acid synthase and IRS-1 protein expression (645).
SOCS-3	CT-1 increases SOCS-3 protein and mRNA expression in 3T3-L1 adipocytes (194, 645).
Endothelin-1	CT-1 increases ET-1 gene expression in and secretion from vascular endothelial cells (243).
Vascular Endothelial Growth Factor	CT-1 induces a 3-fold increase in VEGF production in human visceral adipocytes (453).

1. *Cardiotrophin-1 Signaling*

CT-1 causes pleiotropic biological responses through the LIFR complex, consisting of the gp130/LIFR- β heterodimer (65). The signaling pathway downstream from gp130 is reported to consist of at least three distinct pathways: JAK/STAT, p42/44 MAPK or ERK1/2, and P13K/Akt (65, 199, 289). Stimulation of the JAK/STAT pathway results in the tyrosine phosphorylation of STAT-3, causing its dimerization and translocation to the nucleus where it can activate target genes (6, 584). Activation of MAPK causes the threonine phosphorylation and activation of NF-IL6, a transcription factor involved in cytokine signal transduction (362). Finally, activation of P13K results in the phosphorylation of Akt and the pro-apoptotic gene BAD (289).

The protective effect of CT-1 is likely to be dependent upon its ability to activate the p42/44 MAPK pathway (52, 309, 446, 497). Although there is agreement on the effects mediated through MAPK, opinions regarding the actions mediated by JAK/STAT vary (65). According to the findings of Sheng et al. (497), Latchman explains that the phosphorylation of the STAT-3 transcription factor was entirely unaffected by inhibition of the MAPK pathway and, therefore, is likely to mediate the hypertrophic effect of CT-1 (299). In line with this statement, the majority of works propose that the activation of STAT-3 promotes myocardial hypertrophy (283, 284, 446, 497). However, according to Takahashi and colleagues (537) the major pathway responsible for the hypertrophic response to CT-1 is MEK-5/ERK-5. Furthermore, the JAK/STAT pathway appears to also be involved in the beneficial mechanism that transduces the protective signal against doxorubicin-induced and postpartum cardiomyopathy, protecting cardiomyocytes from ischemic and oxidative stress, promoting myocardial angiogenesis and tissue oxygenation during reperfusion, and controlling interstitial collagen metabolism with a reduction in cardiac fibrosis (204, 205, 283, 366, 367).

Finally, the P13K/Akt pathway appears to, along with the p42/44 MAPK pathway, promote cardiac myocyte survival against apoptosis (368). Neither MEK1, p42/44 MAPK nor P13K/Akt pathway activation alone is sufficient to induce CT-1 mediated cardioprotection against non-ischemic stimuli and re-oxygenation (53, 321). Thus, the MAPK and P13K/Akt pathways may cooperate to produce the cardioprotective effects of CT-1 (53, 65, 289, 321). See figure 3 for an overview of cardiotrophin-1 signaling (65).

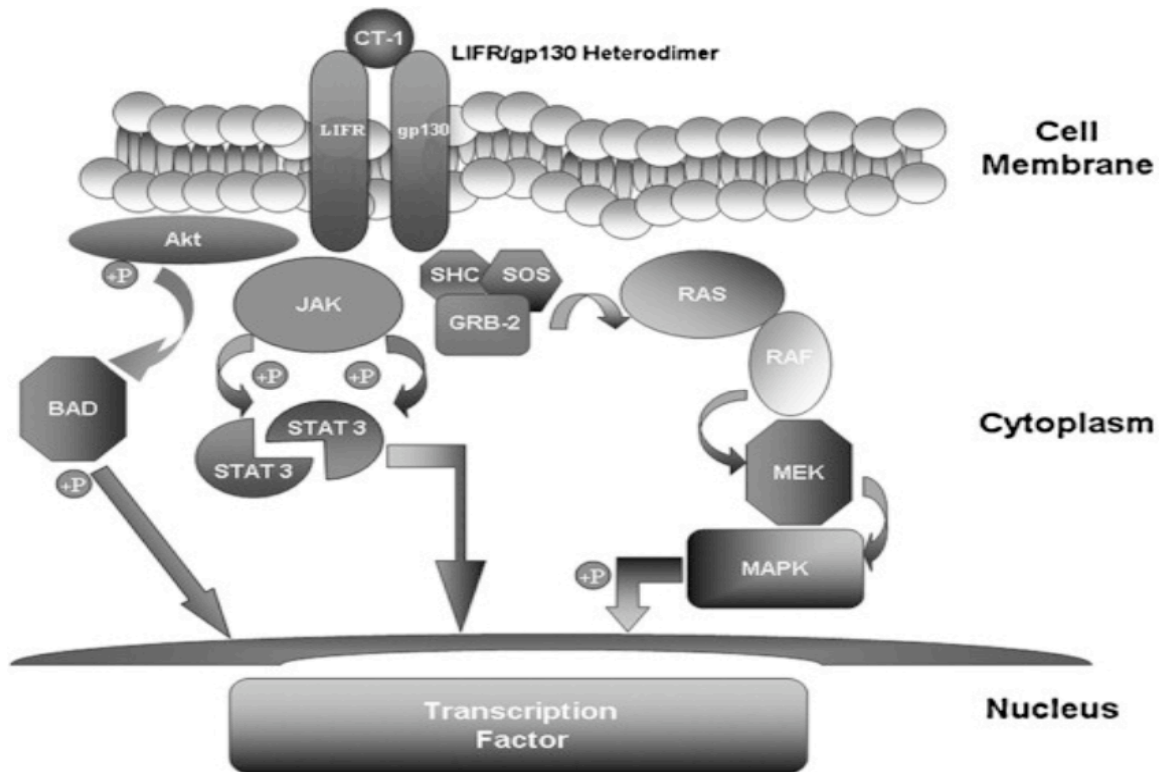


Figure 3. Cardiotrophin-1 signaling. Structure of the CT-1 receptor, made up of the LIFR/gp130 heterodimer and the intracellular pathways activated by CT-1.

2. CT-1 and the Heart

As stated previously, cardiotrophin-1 has two well-documented effects on the heart: myocardial hypertrophy and cardioprotection/cardiac myocyte survival. CT-1 was first isolated as a factor capable of inducing cardiac myocyte hypertrophy, one of the most important adaptive responses of the heart and a central feature of many cardiac diseases in man (65). Cardiomyocyte hypertrophy eventually leads to cardiac muscle failure at its latest stages. The original report on CT-1 from Pennica et al. (422) showed that CT-1 was a potent inducer of hypertrophy, with activity being detected at concentrations of 0.1 nM or lower.

Further *in vitro* studies showed that the hypertrophy induced by CT-1 was distinct in terms of cell morphology and gene expression pattern from that induced by, for example, α -adrenergic stimulation (606). CT-1 promotes an increase in cardiac cell size caused by an increase in cell length (sarcomeres in series) without a significant change in cell width (sarcomeres in parallel) (606). Studies also indicate that CT-1 induces a

distinct gene expression pattern as it up-regulates the atrial natriuretic peptide gene, a marker activated during hypertrophy *in vivo*. However, CT-1 does not affect skeletal α -actinin or myosin light chain-2 synthesis (606). This is indicative of volume overload-induced hypertrophy as opposed to the pressure overload-induced hypertrophy that is characteristic of α -adrenergic stimulation (606). These findings led Wollert and colleagues (606) to conclude that CT-1-induced hypertrophy is closely related to the volume overload-induced hypertrophy that occurs during valvular insufficiency and results in irreversible loss of cardiac function in humans. Jin et al. (242) further confirmed the hypertrophic effects of CT-1 through experiments *in vivo*. Chronic administration of CT-1 to mice caused a dose-dependent increase in both heart weight to body ratio and ventricular weight to body ratio (242). Based on this evidence, CT-1 can clearly induce cardiac hypertrophy both *in vivo* and *in vitro* (242, 299, 606).

Cardiac muscle cell survival plays a critical role in maintaining the normal function of the heart. Adult cardiac muscle cells are fully developed and no longer differentiate. Thus, they have lost their proliferative capacity, and a heart injury might result in scarring and an eventual decrease in global cardiac function (65). The literature has widely shown that CT-1 has a cardioprotective effect. Sheng and colleagues (497) demonstrated that treatment with CT-1 was able to enhance the survival of cultured neonatal rat cardiac myocytes. Furthermore, Stephanou et al. (526) found that pretreatment with CT-1 was able to protect cultured neonatal cardiac myocytes against subsequent exposure to either heat shock or simulated ischemia/hypoxia. Similarly, CT-1 was also able to enhance levels of heat shock proteins hsp70 and hsp90, whose over-expression has been shown to protect cardiac myocytes against both thermal and ischemic stress (93, 195, 196). CT-1 appears to exert its cardioprotective effects by minimizing the degree of programmed cell death (apoptosis) that is induced by serum removal or by thermal and ischemic stress (52, 53, 497, 526). More recent studies have further documented, both in neonatal and adult cardiac cells, the cardioprotective effects of CT-1 against ischemia, when added both prior to and after hypoxic stimulus (52, 309). In addition, CT-1 promoted cardiac myocyte survival against non-ischemic death stimuli, such as angiotensin II and hydrogen peroxide (H_2O_2) (321). Therefore, myocardial CT-1 expression is a classic example of a cardiac compensatory mechanism that can be helpful

of harmful. CT-1 expression is anti-apoptotic and promotes hypertrophy, both of which may initially be beneficial. However, CT-1 appears to be involved in the induction of pathological hypertrophy known to have an adverse effect on left ventricular systolic function (56).

Due to these different effects on the heart, CT-1 may be a relevant marker of disease and may play a role in the pathological changes typical of such cardiovascular diseases as hypertension, congestive heart failure, and ischemic heart disease (65). Hypertensive heart disease is largely characterized by the presence of left ventricular hypertrophy (LVH) (517). Systemic hypertension places a mechanical overload on the left ventricle and activates several stress pathways that induce an increase in left ventricular mass (65). It has been proposed that some of these pathways involve gp-130 dependent ligands (420). Furthermore, a recent study indicates that IL-6-related cytokines may participate in the development of hypertensive LVH (285). Concentrations of cardiotrophin-1, a member of the IL-6 family of cytokines, are elevated in humans with both treated and untreated hypertension as compared to normotensive individuals (320, 421). Moreover, in hypertensives, CT-1 levels are higher in those with LVH as opposed to those with normal ventricular thickening (320). Lopez and colleagues (319) further report that plasma levels of CT-1 in subjects with essential hypertension are correlated to inappropriate left ventricular mass (ILVM), defined as left ventricular mass/predicted left ventricular mass > 128%. In treated hypertensives, normalization of CT-1 concentrations is associated with a regression of ILVM/LVH, whereas an increase in CT-1 levels results in persistent ILVM/LVH (171, 319).

Congestive heart failure is a condition in which the heart's ability to deliver oxygen rich blood to the body is not sufficient to meet the body's needs (65). Both atrial and ventricular gene expression of CT-1 is increased in animal models of CHF (11, 245). Also, in animal models, CT-1 expression precedes the development of the pathological hypertrophy that characterizes CHF (225). In humans with congestive heart failure circulating concentrations of CT-1 are elevated (541). However, the exact role of CT-1 in the pathophysiology of CHF or as a marker of CHF remains unclear (65). Plasma concentrations of CT-1 increase with the severity of CHF and CT-1 levels are significantly higher in hearts explanted from patients with end-stage heart failure as

opposed to donor hearts (644). These explanted hearts expressed 142% higher levels of CT-1 mRNA and 68% higher CT-1 protein concentrations (644). In patients with dilated cardiomyopathy, characterized by volume overload, CT-1 concentrations correlate with left ventricle mass index, suggesting a role for CT-1 in left ventricular remodeling and left ventricular hypertrophy (562). Brain natriuretic peptide (BNP) is a marker for overt CHF (65). Ventricular CT-1 expression is elevated, while ventricular BNP gene expression is not augmented in early stage left ventricular dysfunction (244). These findings suggest that CT-1 is a biomarker for detecting early ventricular dysfunction as compared to BNP (65). Furthermore, Tsutamoto et al. report on the additional prognostic value of CT-1 alone or combined with BNP in patients with CHF (561).

Ischemic heart disease is the most common cause of mortality worldwide (65). Elevated serum concentrations of CT-1 are clearly correlated with the degree of left ventricular systolic dysfunction and are observed in individuals with unstable angina, acute myocardial infarction and heart failure (542-544). In addition, CT-1 expression was elevated in the post-myocardial infarction heart from 24 hours to 8 weeks (151). Evidence suggests that CT-1 has beneficial effects during the early phases of post-MI wound healing. These effects include: promoting myocardial cell survival, inducing hypertrophy of remaining myocytes, and inducing proliferation and migration of fibroblasts from adjacent viable myocardium (outside the infarct zone). In this way, CT-1 can help reduce myocyte death and improve ventricular performance (150, 151). However, in the chronic post-MI stages, CT-1 may actually contribute to the deterioration of ventricular function due to the stimulation of ventricular hypertrophy (65). Therefore, chronically increased synthesis and release of CT-1 could accelerate contractile dysfunction, whereas acute synthesis could preserve contractility (643). The role of CT-1 as a marker and as a cause of structural and functional changes typical of advanced cardiovascular disease is becoming well documented. CT-1 has a wide variety of functions that sometimes have opposite results. Thus, at this time it is difficult to say whether CT-1 is a favorable or adverse molecule in the context of cardiovascular disease (65).

3. *CT-1, Adipose Tissue, and Metabolic Disease*

Two recent investigations have explored the effect of cardiotrophin-1 in adipose tissue and metabolic disease (365, 645). Zvonic and colleagues (645) sought to determine the role of CT-1 on adipocyte physiology. CT-1 administration results in a dose- and time-dependent activation and nuclear translocation of STAT-1, -3, -5A and -5B as well as ERK-1 and -2 MAPKs in 3T3-L1 adipocytes in vitro. Furthermore, acute CT-1 administration was able to activate STAT-1 and -3, as well as ERK-1 and -2 in the epididymal fat pads of C57B1/6J mice in vivo (645). Acute treatment of CT-1 in 3T3-L1 adipocytes resulted in the induction of SOCS-3 mRNA as well as a transient down-regulation of PPAR- γ mRNA. These effects were independent of MAPK activity, providing evidence that they involve the same signaling pathways that induce CT-1's hypertrophic effects. The ability of CT-1 to regulate PPAR- γ and SOCS-3 may prove to be interesting in its relation to affecting adipocyte signaling and metabolism (645). Chronic administration of CT-1 to 3T3-L1 adipocytes resulted in decreased protein levels of IRS-1 after 72 and 96 hours of exposure (645). Based on this result, Zvonic et al. (645) examined the effects of CT-1 on insulin stimulated glucose uptake in 3T3-L1 adipocytes. One hour pretreatment with CT-1 did not significantly inhibit glucose uptake. Following a 24 and 96 hour pretreatment, insulin-stimulated glucose uptake was significantly decreased. The 96-hour pretreatment specifically resulted in a 4-fold decrease as compared to control cells (645). Therefore, the down-regulation of IRS-1 expression by CT-1 could be a possible marker of impaired insulin sensitivity in CT-1 treated adipocytes. The observation that chronic CT-1 exposure results in a statistically significant decrease in insulin-stimulated glucose uptake supports the hypothesis that CT-1 may be a mediator of impaired insulin sensitivity (645). Elevated circulating levels of CT-1 are found in patients with ischemic heart disease and valvular heart disease (151). Clinical aspects of these diseases are tightly linked to obesity and type 2 diabetes (450, 518). Therefore, CT-1 may serve as a link between obesity-related complications and cardiovascular disease (645).

Natal and colleagues (365) sought to test whether adipose tissue expresses CT-1 and whether CT-1 expression can be modulated as well as to compare serum CT-1 concentrations in subjects with and without metabolic syndrome as diagnosed by the

NCEP ATP III criteria. These investigators found that both mouse and human adipocytes express cardiotrophin-1. Moreover, in 3T3-L1 adipocytes, CT-1 expression progressively increases along with differentiation time from preadipocyte to mature adipocyte. In other words, mature adipocytes express higher levels of CT-1 than do undifferentiated cells. The analysis of various murine tissues including the heart, thymus, spleen, kidney, pancreas, liver and adipose tissues confirmed this novel finding that adipose tissue is an important source of CT-1. In fact, CT-1 expression in fat tissue exceeded by three-fold that found in other tissues tested. However, it has not yet been determined whether CT-1 release by adipose tissue is due to resident cells (macrophages, etc.) rather than adipocytes (365).

Furthermore, Natal et al. (365) screened glucose and some cytokines/neurohumoral factors implicated in obesity and inflammation for their ability to induce CT-1 expression. All tested stimuli positively increased CT-1 expression in 3T3-L1 cells, however glucose significantly increased adipocyte-derived CT-1 mRNA and protein expression in a dose-dependent manner. This effect of glucose could account for the elevated circulating CT-1 concentrations observed in individuals with hyperglycemia and metabolic syndrome (MetS) (365).

Natal and colleagues (365) observed significantly elevated serum CT-1 concentrations in obese subjects and in individuals with hyperglycemia and MetS as opposed to normal-weight controls and those with normal fasting blood glucose concentrations. Furthermore, in the entire population of individuals both with and without MetS, CT-1 concentrations positively correlated with glucose but not with blood pressure, triglycerides, HDL-C or BMI. Similarly, CT-1 concentrations were significantly higher in subjects with hyperglycemia (fasting glucose > 100 mg/dL), but not significantly different in patients with hypertension, hypertriglyceridemia or low HDL-C (365). In other words, increased serum levels of CT-1 were observed in individuals with MetS, particularly in obese and diabetic subjects, which could be explained by overexpression of the cytokine from adipose tissue (365). The altered release of cytokines by adipose tissue in obesity may have negative effects on the cardiovascular system (90, 126, 554). The novel observation from Natal and colleagues (365) that adipose tissue expresses CT-1, a cytokine with demonstrated cardiovascular

and metabolic actions, presents the possibility that CT-1 may play a pathophysiological role in MetS, serving as a link between obesity-related complications and cardiovascular disease. Moreover, glucose-induced adipose tissue expression of CT-1 may further explain the relationship between obesity and insulin resistance in MetS (365).

4. CT-1 and Reactive Oxygen Species/Oxidative Stress

Several recent studies have demonstrated a direct relationship between cardiotropin-1 and reactive oxygen species (ROS)/oxidative stress (21, 482). It has previously been discussed that CT-1 activates the Jak/STAT signaling cascade as well as the ERK/Akt (or protein kinase B) pathways, which are known to be involved in the regulation of cell proliferation and cytoprotection (289, 497). It has also recently been demonstrated that CT-1 activates NF- κ B and p38, which may partially mediate the cytoprotective and anti-apoptotic effects of CT-1 in cardiomyocytes (91). The Jak/STAT, ERK and NF- κ B signaling pathways have previously been shown to be regulated by intracellular reactive oxygen species. Thus, CT-1 may exert its biological effects through an elevation of ROS, which act as signaling molecules in CT-1 induced signal transduction cascades (238, 482-484, 511).

Sauer et al. (482) investigated the involvement of ROS in CT-1 mediated signaling cascades in cardiomyocytes differentiated from murine embryonic stem cells. This group found that upon stimulation of cells with CT-1, intracellular ROS levels increased rapidly via the elevated activity of NADPH-oxidase, which is regulated by PI3-kinase. These reactive oxygen species are then utilized as signaling molecules to ensure the proper functioning of the CT-1-induced signaling pathways (482). Furthermore, Sauer and colleagues (482) found that CT-1's stimulation of cardiac cell proliferation was regulated by ROS, as in the presence of the free radical scavenger vitamin E, the stimulation of cardiomyocyte proliferation was abolished. In summary, CT-1 elicits an increase in intracellular ROS via NADPH-oxidase, regulated by PI3-kinase (482, 484). ROS generated by NADPH-oxidase in response to stimulation with CT-1 may be utilized to initiate and maintain CT-1-mediated signaling cascades (482).

The properties of CT-1 as a cardioprotective and hypertrophic cytokine in stress conditions predict up-regulation during cardiac diseases that are characterized by an

environment of hypoxia, inflammation and oxidative stress (21). Ateghang and colleagues (21) have recently shown that CT-1 expression is regulated by reactive oxygen species and hypoxia in differentiating mouse embryonic stem cells. ROS and hypoxia use a common HIF-1-regulated signaling pathway that results in increased CT-1 expression. HIF-1 is a heterodimer composed of the constitutively expressed β -subunit and the oxygen-dependent α -subunit (HIF-1 α) that is stabilized under hypoxic conditions (211).

Treatment with the antioxidant vitamin E down-regulated CT-1 as well as HIF-1 α , indicating regulation by ROS endogenously generated in differentiating embryonic stem cells (21). Treatment of these same cells with the pro-oxidant hydrogen peroxide resulted in a dose dependent increase in CT-1 protein expression. Under the same experimental conditions, increased protein and mRNA expression of HIF-1 α was also observed, thus corroborating the idea of parallel regulation of CT-1 and HIF-1 α by pro-oxidants. However, statistical significance was achieved at earlier time points for HIF-1 α expression compared with CT-1 expression, suggesting that HIF-1 α precedes CT-1 expression (21). This hints that the up-regulation of CT-1 by ROS is mediated by HIF-1 signaling. The up-regulation of CT-1 mRNA and protein was completely abolished in HIF-1 α -deficient embryonic stem cells. This further supports the notion that ROS and hypoxia regulated CT-1 expression is mediated by HIF-1 (21). Furthermore, pro-oxidants appear to increase the activity of ROS generating NADPH-oxidase thereby providing a feed-forward loop of increased ROS generation even in the absence of external pro-oxidants. This, in turn, will help to induce an increased expression of CT-1 via HIF-1. On the other hand, an inhibition of NADPH-oxidase significantly reduced the increase in CT-1 expression following treatment with pro-oxidants, thus indicating that NADPH-oxidase derived ROS are involved in up-regulation of CT-1 by pro-oxidants and hypoxia (21).

Hypoxia is a pathophysiological situation occurring during conditions where increased expression of CT-1 has been reported, such as angina pectoris, myocardial infarction and heart failure (56, 65, 151). It has recently been demonstrated that hypoxia is associated with increased ROS generation produced either through the mitochondrial respiratory chain or NADPH-oxidase (605). Therefore, CT-1 is associated with hypoxic

conditions associated with increased generation of ROS. Based on the studies from Sauer et al. (482) and Ateghang et al. (21), it appears that CT-1 elicits an increase in ROS via NADPH-oxidase regulated by PI3-kinase. This elevation in ROS, in turn, induces an up-regulation of CT-1 mediated by HIF-1 signaling (21). Thus, it is possible that CT-1 and ROS regulate one another through a positive feedback loop initiated by pathophysiological hypoxia.

Fibroblast Growth Factor-21

Fibroblast growth factor-21 was first identified in 2000 as a secreted protein preferentially expressed in the liver (374). The fibroblast growth factor family consists of 22 members, divided into 7 subfamilies based on structural similarities and modes of action (438). A majority of FGFs serve as paracrine factors regulating cell growth and differentiation, including angiogenesis and transformation (229). Members of the FGF-19 subfamily, including FGF-21 and FGF-23, differ in that they have no or very small mitogenic effects and they exert important metabolic effects via systemic endocrine mechanisms (374, 475, 506, 615). The three members of the FGF-19 subfamily share approximately 30% amino acid sequence homology (267). FGF-19 subfamily members regulate diverse physiological processes that are not affected by other FGFs. These metabolic activities include the regulation of lipid and carbohydrate metabolism, as well as bile acid, phosphate, calcium and vitamin D homeostasis (208, 268, 324, 505, 552). FGF-21 is predominantly expressed in the liver and has beneficial effects in various animal models of obesity and metabolic disease (89, 268, 269). FGF-21 expression in the liver was shown to be under the control of the transcription factor PPAR- α (325). However, FGF-21 expression is rather plastic and can be induced by PPAR- γ activation in adipocytes and muscle cells (233, 357, 358, 580). The human FGF-21 gene is located on chromosome 19 and encodes a 209 amino acid protein. Human and mouse FGF-21 share a 75% amino acid sequence identity (267). Results from recent studies show that FGF-21 expression and regulation in human subjects is considerably different than that observed in animal models, thus provoking discussion of the possible clinical significance of this cytokine (73, 89, 268, 269, 308, 636).

1. FGF-21 Signaling

Fibroblast growth factors mediate their action via a set of membrane-bound FGF receptors (FGFR). Upon binding, FGF-21 stimulates tyrosine phosphorylation of FGFRs, which appears to be critical to FGF-21 activity. This leads to the activation of a number of downstream signals including MAPKs, RAF1, AKT1, and STATs. The same effects can be caused by archetypal FGFs such as FGF1, providing evidence that FGF21 signals through activation of a conventional FGFR-mediated pathway (268, 357, 381, 592). However, FGFs cannot interact with FGFRs directly as they require a co-factor to bind and activate FGFR signaling. For most, this co-factor is heparin/heparin sulfate (169, 475). FGF-21 is unique in that it does not bind or require heparin for its bioactivity, fails to directly interact with soluble FGFRs and shows an activity profile restricted to adipose and pancreatic cells (169, 268, 357, 381). The weak heparin sulfate binding affinity prevents FGF-21 from being captured in the extracellular matrix, thus allowing it to function as an endocrine factor (169, 349).

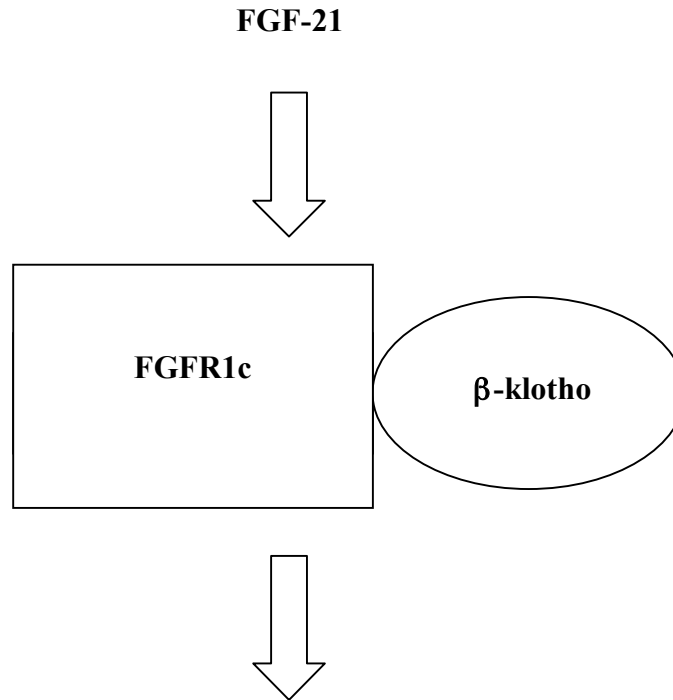
FGF-21, along with the other members of the FGF-19 subfamily, require the presence of specific transmembrane proteins from the klotho family for FGFR binding and activation (288, 566). β -klotho, a protein that shares 41% amino acid identity with klotho, is the critical component of the FGF-21 receptor complex (288, 381). β -klotho is a type I transmembrane protein whose function is not well understood. A recent report links this molecule to cholesterol and bile acid metabolism; however, the exclusive expression of β -klotho in the pancreas, liver and adipose tissue suggests a broader biological role (226, 227, 267, 286).

An investigation in murine 3T3-L1 cells showed that β -klotho forms a preformed complex with FGFR1 or FGFR2, which is then activated by FGF-21 binding (266). Two studies concluded that the carboxy-terminus region of FGF-21 is essential for β -klotho binding, while the amino-terminus is important for FGFR activation (344, 624). Furthermore, several reports suggest that the c-receptor splice isoforms of FGFR1-3 exhibit an affinity to β -klotho and most likely act as endogenous receptors for FGF-21 (266, 287, 381). FGF-21 was shown to be bioactive in murine BaF3 cells stably expressing both β -klotho and FGFR1c or FGFR3c, but not in those that do not express any endogenous FGFRs (267). Similarly, cells lacking β -klotho do not respond to FGF-

21 and the introduction of β -klotho to these cells confers FGF-21 responsiveness (266). Moreover, in 3T3-L1 cells, transient overexpression of FGFR4 resulted in interaction with β -klotho and the binding of FGF-19 and FGF-21 (267).

These results together suggest that FGF-21 may interact with different FGFRs in different cellular environments as long as β -klotho stimulates receptor activation (475). β -klotho is almost exclusively expressed in the pancreas, liver and adipose tissue (227). The main effects of FGF-21 have been observed in the adipose tissue and pancreas, however, no direct effects of FGF-21 have been observed in the liver (475). This may be due to the fact that the liver predominantly expresses FGFR4. Although both FGF-19 and FGF-21 can bind to the β -klotho-FGF-4 complex, only interactions with FGF-19 result in sufficient receptor activation (287). On the other hand, Kharitonov and colleagues (267) explain that FGF-21 likely does have an effect on liver-derived cells, given the profound impact of FGF-21 on lipid metabolism in animals. FGFR1 is the most abundant receptor in 3T3-L1 cells and in white adipose tissue. Thus, in adipose tissue, it is likely that the main functional receptors of FGF-21 are preformed β -klotho-FGFR1c complexes (266, 287) (see figure 4).

However, although the initial steps in FGF-21 signaling are becoming better characterized, the affected downstream target genes remain to be defined (475). Furthermore, molecular specifics about the initial FGF-21 mechanism of action need to be further clarified (267). In native conditions, FGF-21 functions through FGFR1 and FGFR2 in adipocytes and pancreatic cells. Multiple splice isoforms of these receptors have been detected, and it is currently unclear which particular variants serve as the specific FGF-21 receptors within the FGF-21/FGFR/ β -klotho complex (268, 381, 592). Also, no FGFR4 and insignificant FGFR3 levels were detected in 3T3-L1 adipocytes (357). Thus, it remains unclear whether FGF-21 can signal through these FGFRs when they are present at elevated levels (267).



Carbohydrate and Lipid Metabolism

Figure 4. FGF-21 signaling in adipose tissue. The interaction of β -klotho and FGFR1 stimulates receptor activation. FGF-21 binds to the β -klotho-FGFR1 complex to induce changes in carbohydrate and lipid metabolism.

2. FGF-21 in Animals

Mounting evidence from animal-based studies suggests FGF-21 as a potent metabolic regulator with multiple beneficial effects on obesity and diabetes (355, 456). Kharitonov and colleagues (268) first identified these effects in 2005. FGF-21 stimulated insulin-independent glucose uptake through increasing GLUT-1 expression in murine 3T3-L1 adipocytes in vitro. Furthermore, FGF-21 administration in *ob/ob* and *db/db* mice lowered blood glucose and triglyceride levels, while transgenic mice that overexpress FGF-21 were protected against diet induced obesity and insulin resistance. Four hours after the administration of FGF-21, GLUT-1 mRNA upregulation was observed in the white adipose tissue, but not in the muscle, liver, kidney, or brain of *ob/ob* mice. Importantly, FGF-21 did not induce mitogenicity, hypoglycemia, or weight gain at any dose tested in diabetic, obese or healthy animals or when overexpressed in transgenic mice (268).

Several studies employing murine models of diet-induced obesity found that FGF-21 administration can reduce body weight by up to 20%, increase energy expenditure and reverse insulin resistance and hepatic steatosis (89, 612). Coskun and colleagues (89) reported that two week systemic administration of FGF-21 in diet-induced obese and *ob/ob* mice resulted in a 20% reduction in body weight predominantly due to a reduction in adiposity. Also, via transcriptional and blood cytokine profiling, FGF-21 treated animals demonstrated increased energy expenditure and fat utilization and reduced hepatosteatosis. In addition, FGF-21 exhibited the ability to ameliorate insulin and leptin resistance, enhance fat oxidation and suppress de novo lipogenesis in the liver (89). Recently, Xu et al. (612) reported similar findings in diet-induced obese mice. FGF-21 dose dependently reduced body weight and fat mass in these animals due to marked increases in total energy expenditure and physical activity levels. Administration of FGF-21 reduced blood glucose, insulin, and lipid levels and reversed hepatic steatosis. FGF-21 also dramatically improved hepatic and peripheral insulin sensitivity in both lean and diet-induced obese mice independently of reductions in body weight and adiposity (612). The findings of these two studies are quite similar, yet they do have their differences. Coskun and colleagues (89) reported a reduced respiratory quotient that is indicative of a preferential utilization of fat as an energy store. Xu et al. (612) found similar reductions in body weight (~20%) via decreased adiposity and increased energy expenditure. However, no changes in respiratory quotient were observed, suggesting that there were no alterations in fuel selection between fatty acids and carbohydrates. Instead these mice demonstrated a significant increase in physical activity (612).

FGF-21 caused a dramatic decline in fasting plasma glucose, triglycerides, insulin and glucagon when administered for 6 weeks to diabetic rhesus monkeys (269). FGF-21 administration also led to beneficial changes in lipid profiles including the lowering of LDL-C and elevation of HDL-C and the induction of small but significant weight loss. Of significant importance from a safety standpoint, hypoglycemia was not observed at any point after FGF-21 administration (269).

The cellular mechanisms through which these effects are elicited are not entirely clear. As mentioned earlier, in murine adipocytes in vitro FGF-21 increases glucose uptake by an upregulation of GLUT-1 (268). However, it is unlikely that an upregulation

of GLUT1 is the main mechanism through which FGF-21 exerts its insulin sensitizing effects (475). The enhancement of GLUT-1 following FGF-21 administration in mice is modest and limited to adipose tissue (268). Also, an upregulation of GLUT-1 does not explain the FGF-21-induced amelioration of dyslipidemia or weight reduction (475). FGF-21 inhibited pancreatic glucagon secretion and increased islet insulin content and glucose-induced insulin release by increasing islet survival through a protection from glucolipotoxicity and cytokine-mediated apoptosis (592). Yet, these effects on islet survival and β -cell function most likely are not the primary mechanism, as they cannot explain the reductions in weight or hepatosteatosis (475). Furthermore, considering the results from Coskun and colleagues (89), the anti-obesity effect of FGF-21 is probably not mediated via changes in circulating factors since out of 67 studied hormones and cytokines, FGF-21 treatment only led to a significant reduction in leptin and insulin. Based on the contrasting findings from Coskun et al. (89) and Xu et al. (612) regarding whether FGF-21 administration affects physical activity, it is still unclear whether FGF-21 increases locomotor activity or not. This should be addressed in future studies as it could provide a mechanism through which FGF-21 exerts its insulin sensitizing and anti-obesity effects in animals (475).

In addition to its insulin sensitizing effects in animals, FGF-21 also appears to mediate the physiological response to food deprivation/starvation and to be a primary factor in initiating the production of ketone bodies (24, 223, 456). As noted earlier, FGF-21 seems to be under the control of PPAR- α in the liver, which is activated during starvation (24, 223). The activation of PPAR- α (via starvation, fibrates or ketogenic diet) in male mice directly results in an increase in circulating FGF-21 concentrations, which promotes lipolysis in adipose tissue. Subsequently, fatty acids are taken up by the liver and converted into ketone bodies, which are crucial energy sources during fasting and starvation (24, 223). Also, Inagaki and colleagues (223) reported that FGF-21 reduces physical activity and promotes torpor, a short-term hibernation-like state of regulated hypothermia that conserves energy. These findings are in direct contrast to previous reports that FGF-21 increases energy expenditure and physical activity (89, 612). However, these contrasting findings could be due to the fact that they were found in response to starvation, as opposed to data from animal models of obesity/energy excess.

Thus, in states of energy excess FGF-21 appears to induce an insulin sensitizing and anti-obesity effect, while in states of starvation/energy deficit FGF-21 promotes energy conservation through ketogenesis and torpor.

As mentioned previously, recent data show that FGF-21 expression can be induced not only in the liver, but also in adipose tissue (358, 580, 636). This induction is most likely regulated by PPAR- γ activity. Wang and colleagues (580) identified an amino acid within helix 7 of the PPAR- γ ligand binding domain that is required for the ability of PPAR- γ to activate a novel group of adipocyte genes, including FGF-21. Furthermore, exposure of 3T3-L1 adipocytes to the PPAR- γ agonist troglitazone resulted in rapid induction of FGF-21 mRNA expression. These results were confirmed by a series of FGF-21 gene promoter/luciferase reporter assays. It was thus determined that PPAR- γ directly regulates expression of FGF-21 (580). Moreover, Muise et al. (358) sought to identify potential secreted proteins regulated by PPAR- γ in diabetic animal models. This group identified 33 genes coding for secreted proteins regulated by PPAR- γ agonists in epididymal white adipose tissue of *db/db* mice, one of which was FGF-21 (358). This indicates that FGF-21 gene expression results from PPAR- γ activation in adipose tissue. Expression of FGF-21 in vivo was also found to be regulated by PPAR- γ , as PPAR- γ agonist treatment in *db/db* mice resulted in a 2-3 fold elevation of FGF-21 mRNA in adipose tissue coincident with an elevation of plasma FGF-21 concentrations (358). Lastly, Zhang and colleagues (636) observed that chronic treatment with the PPAR- γ agonist rosiglitazone caused a dramatic induction of FGF-21 production in 3T3-L1 adipocytes. These results suggest a role for FGF-21 in mediating the anti-dibetic effects of PPAR- γ agonists (358).

In addition, it appears that FGF-21 also regulates PPAR- γ expression. Continuous treatment of 3T3-L1 cells with FGF-21 induces an increase in PPAR- γ protein expression. Treatment of cells with FGF-21 and the PPAR- γ agonist rosiglitazone in combination leads to a pronounced expression of GLUT-1 and a marked stimulation of glucose uptake. Treatment with either FGF-21 or PPAR- γ alone did not significantly increase glucose transport (357). These results reveal a novel synergy between FGF-21 and PPAR- γ in regulating glucose homeostasis. This synergy demonstrated a marked

functional interplay between FGF-21 and PPAR- γ pathways. In summary, FGF-21 stimulation of 3T3-L1 adipocytes elevates PPAR- γ expression and the PPAR- γ agonist rosiglitazone modulates FGF-21 action, sensitizing its ability to activate/induce tyrosine phosphorylation of FGFR-2 (357). These non-human data suggest that FGF-21 and PPAR- γ regulate one another as a possible protective pathway in diabetic and obese states.

3. FGF-21 in Humans

There are no published data to date regarding the effects of FGF-21 in humans *in vivo*. Only studies investigating the effects in human cells *in vitro* and cross-sectional analysis of FGF-21 concentrations in various subpopulations show that the effects of FGF-21 in humans do not appear to be consistent with those observed in animal models (18, 73, 111, 161, 307, 308, 636). For example, murine FGF-21 seems to promote lipolysis in 3T3-L1 adipocytes (223, 268). However, FGF-21 has no effects on basal lipolysis, and actually attenuates hormone-stimulated lipolysis in primary cultures of human adipocytes. It must be noted though that these effects were only elicited at supraphysiological concentrations (18). Based on these data, FGF-21 may have a beneficial effect on insulin sensitivity in man. The increased release of fatty acids into circulation is a well-established factor underlying the development of insulin resistance (16). Although it remains to be shown, the FGF-21 mediated attenuation of lipolysis could result in reduced levels of circulating free fatty acids *in vivo* thus positively influencing insulin sensitivity (475).

Several recent investigations have assessed circulating concentrations of FGF-21 in various human cohorts (73, 111, 161, 307, 308, 636). Zhang et al. (636) reported that serum FGF-21 levels were significantly higher in overweight/obese subjects than in lean individuals. Serum FGF-21 concentrations also correlated positively with BMI, waist circumference, waist-to-hip ratio, fat percentage, fasting insulin, HOMA and triglycerides and negatively with HDL-C, QUICKI and adiponectin after adjusting for age and BMI. Moreover, logistic regression analysis showed an independent association between serum FGF-21 levels and the metabolic syndrome. The increased risk of the metabolic syndrome associated with elevated serum FGF-21 was over and above the effects of

individual components of MetS (636). However, interestingly, no significant difference in serum FGF-21 levels was observed between non-diabetic and diabetic subjects. This study illustrates an association between elevated circulating concentrations of FGF-21 and metabolic complications, which directly conflicts with data gathered from animal models. The paradoxical increase in serum FGF-21 in obese individuals may possibly be explained by a compensatory response or a resistance to the protein similar to that seen with leptin (636).

Li and colleagues (308) investigated whether plasma FGF-21 levels were different in patients with type 2 diabetes (T2DM) or diabetic ketosis (T2DK) and in sex- and age-matched normal controls. Plasma concentrations of FGF-21 were markedly higher in patients with T2DK and T2D than in controls. Increasing plasma concentrations of FGF-21 were independently and significantly associated with T2DM and T2DK even after controlling for anthropometric variables, blood pressure and lipid profile. Fasting plasma FGF-21 was positively correlated with systolic blood pressure, diastolic blood pressure, HbA_{1c}, HDL-C, and FFA and negatively correlated with fasting plasma insulin and insulin sensitivity. Multiple regression analysis demonstrated that diastolic blood pressure, waist-to-hip ratio and FFA were independent related factors regulating plasma FGF-21 concentrations. These results further suggest that FGF-21 is related to metabolic complications in humans and may play a role in the pathogenesis of type 2 diabetes (308). It must be noted, however, that the findings of this study conflict with the results from Zhang et al. (636) that showed no elevation in FGF-21 concentrations in diabetic subjects. The differences in study design, including subject selection and experimental conditions is likely the contributing factor to this discrepancy.

Chen et al. (73) investigated whether or not plasma FGF-21 levels were different in patients with type 2 diabetes mellitus (T2DM) and non-diabetic controls. Fasting FGF-21 concentrations were significantly higher in patients with T2DM as compared to controls. There were no gender differences and plasma FGF-21 levels remained significantly higher in diabetics after adjustment for age, sex and BMI and after controlling for anthropometric variables and differences in blood pressure and lipids (73). This result is in line with the findings of Li and colleagues (308), yet in contrast to the findings of Zhang and colleagues (636). Furthermore, plasma FGF-21 concentrations

inversely correlated with fasting plasma glucose in simple regression analysis. After multiple regression analysis, fasting plasma glucose, insulin and insulin sensitivity as assessed by HOMA, negatively correlated with plasma FGF-21 levels. These inverse correlations suggest that insulin secretion or action may be an FGF-21 determining factor. Moreover, elevated FGF-21 levels in patients with T2DM may suggest the impairment of FGF-21 signaling in target tissues or the dysregulation in biosynthesis/in response to hyperglycemia or hyperinsulinemia in a diabetic state (73). The results here further suggest a potential role for FGF-21 in the pathogenesis of insulin resistance and T2DM.

The findings of Li and colleagues (307) are a bit different from those discussed previously (73, 308, 636). This group reported that serum FGF-21 concentrations were significantly higher in subjects with impaired glucose tolerance than in individuals with normal glucose tolerance after adjustment for age, gender and BMI. Serum FGF-21 levels correlated positively with alanine aminotransferase, γ -glutamyltransferase, total cholesterol, triglycerides, LDL-C and several parameters of adiposity including BMI, waist circumference, fat percentage and fat mass after adjustment for age. A negative association between FGF-21 and HDL-c was also demonstrated. Multiple stepwise regression analysis showed an independent association of serum FGF-21 with serum triglycerides, total cholesterol and γ -glutamyltransferase. However, unlike the previous three studies discussed, FGF-21 did not correlate with insulin secretion and sensitivity as measured by hyperglycemic clamp and 75-g oral glucose tolerance test (307). Thus, these data demonstrate that serum concentrations of FGF-21 are elevated in subjects with impaired glucose tolerance, however, FGF-21 is not related to insulin secretion and sensitivity. Instead, serum FGF-21 levels in humans appear to be more related to lipid metabolism and early liver injury (307).

Since little information is available on the functions of FGF-21 in humans, Galman and colleagues (161) carried out studies to analyze circulating levels of FGF-21 in various conditions, such as in normal and hypertriglyceridemic humans and during metabolic perturbations induced by fasting, a ketogenic diet, and by fenofibrate (a PPAR- α agonist) treatment. Results demonstrated that FGF-21 circulates in humans at highly variable levels. Specifically, serum FGF-21 varied 250-fold among 76 healthy

individuals and did not relate to age, gender, BMI, serum lipids, or plasma glucose. It should be noted that subjects with high or low FGF-21 concentrations maintained their individual levels when sampled for 25 hours. This indicates that there is an intrinsic variation in FGF-21 metabolism in healthy subjects. Therefore, variation in serum concentrations FGF-21 may not be important for metabolic regulation (161). No increases in serum FGF-21 were observed following a 48 hr fast or ketogenic diet treatment, despite the induction of ketosis. However, fasting for 7 days significantly increased serum FGF-21, yet these levels remained in the normal range. FGF-21 concentrations were also elevated by two-fold in hypertriglyceridemic patients as compared to healthy controls, while fenofibrate treatment lowered serum triglycerides and increased serum FGF-21 (161). In summary, Galman et al. (161) ascertain that there is a large interindividual variation in serum levels of FGF-21 in humans and that hypertriglyceridemia appears to be linked to elevated levels. Prolonged fasting and fenofibrate treatment both increase FGF-21, although ketogenesis can clearly be induced independent of serum FGF-21 concentrations. These results together hint that the physiological roles of FGF-21 in man may be far different from those observed in mice (161).

Interestingly, Dostalova et al. (111) measured plasma concentrations of FGF-21 in patients with anorexia nervosa in order to explore a relationship with anthropometric and endocrine parameters. Plasma FGF-21 concentrations were significantly reduced in anorexia nervosa relative to normal age-matched controls and were positively correlated with BMI, serum leptin and insulin. An inverse relationship to serum adiponectin was reported in both the anorexia nervosa and control groups. No significant relationship was found between FGF-21 and serum ketone bodies, FFA or HOMA. Multiple regression analysis showed that leptin and adiponectin were independent predictors of plasma FGF-21 concentrations (111). Similar to the previous studies discussed that measured FGF-21 concentrations in obese individuals, these results suggest that circulating levels of FGF-21 are strongly related to body weight. Moreover, in line with the results from Galman and colleagues (161) and contradictory to acute fasting animal studies, malnutrition (fasted state) in humans is associated with markedly

reduced levels of FGF-21. These findings further demonstrate that the bioactivity of FGF-21 in humans is different relative to that in rodents.

FGF-21 is primarily believed to act via endocrine mechanisms. Thus, a detailed assessment of tissue expression is lacking in these studies. However, the results of these studies help characterizing the bioactivity of FGF-21 in humans. They show some similarities, but many differences with previously published data in animals, suggesting that the biology of FGF-21 may be quite complex (475). Plasma FGF-21 levels are highly variable in normal weight subjects, yet a significant positive correlation is observed with obesity, the metabolic syndrome and type 2 diabetes (73, 161, 308, 636). This paradoxical increase of FGF-21 concentrations, as compared to animal data, might be a defensive response of the human body to counteract the stress imposed by obesity and metabolic complications. Alternatively, reminiscent of hyperinsulinemia and hyperleptinemia, obesity and metabolic disorders may cause a resistance to FGF-21's actions leading to its compensatory up-regulation (636). Another possibility is that FGF-21 in circulation may undergo truncations in either the amino- or carboxy-terminal region, thereby inactivating the protein.

The elevated levels observed in various clinical conditions in man could be characterized by the presence of a non-functional FGF-21 molecule. These possible mechanisms as to why FGF-21 concentrations seem to be elevated in conditions associated with the metabolic syndrome are of great interest and have yet to be investigated. Therefore, one can only speculate as to whether the increase in FGF-21 is a protective mechanism, an indirect sign of FGF-21 resistance or a phenomenon secondary to reduced plasma clearance, increased ectopic expression or the presence of a truncated, inactive form of the protein in circulation (475).

Adiponectin

Adiponectin, a protein hormone also known as adipoQ or adipocyte complement related protein, is specifically and very highly expressed in adipose tissue (177). Adiponectin is also the most abundantly expressed adipokine in white adipose tissue and circulates in concentrations of 5 to 10 $\mu\text{g/mL}$ in human serum (36, 222). Adiponectin exerts insulin-sensitizing and anti-atherogenic effects (76). The hormone enhances

insulin sensitivity in the muscle and liver and increases free fatty acid (FFA) oxidation in several tissues (155, 618). It also lowers serum FFA, glucose and triglyceride concentrations (155, 177). In circulation, adiponectin forms a variety of multimers such as trimers (low molecular weight – LMW), hexamers (medium molecular weight – MMW) and dodecamers or 18-mers (high molecular weight – HMW) (246). It has been shown that HMW adiponectin is the active form that results in the improvement of insulin sensitivity (136). Two receptors for adiponectin have been identified: adipoR1 and adipoR2 (616). Disruption of both prevents adiponectin binding, resulting in increased triglyceride levels, inflammation and oxidative stress (619). This further supports the role of adiponectin in regulating insulin sensitivity (550).

Clinical studies implicate levels of adiponectin in the pathogenesis of obesity related disease. Unlike most cytokines, adiponectin concentrations are decreased in obesity (14, 81, 594). Plasma concentrations are negatively correlated with BMI (14). Moreover, a longitudinal study in primates demonstrated that adiponectin decreases with weight gain as the animals become obese (217). Conversely, plasma adiponectin levels increase significantly following weight loss (621). Adiponectin levels differ with the distribution of body fat as well (222). Indeed, plasma concentrations exhibit strong negative correlations with intra-abdominal fat mass (81). Visceral, but not subcutaneous, fat stores were reported to be inversely associated with plasma adiponectin levels in healthy women (290). A low waist to hip ratio is associated with elevated circulating levels of adiponectin, independent of body fat percentage (521). Furthermore, plasma adiponectin concentrations are lower in individuals with type 2 diabetes mellitus (216). Adiponectin levels correlate strongly with insulin sensitivity, suggesting a role for low plasma concentration in the development of insulin resistance (523). In a study of Pima Indians, who demonstrate a high incidence of obesity, insulin resistance and type 2 diabetes, individuals with high levels of adiponectin were less likely to develop diabetes (311). Adiponectin concentrations are also reported to be associated with components of MetS. Elevated levels relate to an advantageous blood lipid profile (29, 557). Moreover, adiponectin concentrations are decreased in persons with hypertension, irrespective of the presence of IR (232). A recent study reported that adiponectin levels might serve as a

biomarker for MetS, demonstrating that adiponectin is a key contributor within the pathogenesis of obesity and MetS (601).

Several studies using experimental animal models have studied the metabolic actions of adiponectin. In obese animals, treatment with adiponectin decreases hyperglycemia and plasma FFA levels and improves insulin sensitivity (36). Adiponectin knockout mice exhibit severe insulin resistance in response to a high fat/sucrose diet (332). This model also displays delayed clearance of plasma FFA, elevated levels of TNF- α mRNA in adipose tissue and higher plasma TNF- α concentrations (332). Restoration of adiponectin expression by adenovirus-mediated gene transfer resulted in a reversal of these conditions (281, 332). Furthermore, studies illustrate that adiponectin regulates glucose metabolism and insulin sensitivity through the phosphorylation and activation of the 5'-AMP-activated protein kinase (AMPK) in muscle and liver tissues (551, 617).

Adiponectin also appears to protect against the development of various vascular diseases through its anti-atherogenic function (578). Adiponectin concentrations are reduced in patients with coronary artery disease (216). In addition, adiponectin inhibits TNF- α induced expression of adhesion molecules and the transformation of macrophages into foam cells, both of which are key components of atherogenesis (395, 396). Administration of adiponectin reduces atherosclerotic lesion size by 30% in apolipoprotein E deficient mice, and this coincides with reductions in VCAM-1 and TNF- α expression in the aortic sinus (386). On the other hand, adiponectin-deficiency leads to an increase in vascular lesion area in apolipoprotein E deficient mice (385). Adiponectin knockout mice also exhibit a greater incidence of hypertension when placed on a high salt diet (384). Adiponectin stimulates the intracellular signaling kinases Akt and AMPK, which phosphorylate and activate endothelial nitric oxide synthase (398). It appears that the anti-hypertensive effects of adiponectin are mediated by its ability to increase the production of nitric oxide by endothelial cells (578).

Adiponectin exerts beneficial effects on the heart under pathological conditions (578). Adiponectin knockout mice contract severe cardiac hypertrophy and overexpression of adiponectin will attenuate cardiac hypertrophy in response to pressure overload in wild type and diabetic *db/db mice* (502). In response to angiotensin II

infusion, adiponectin deficient mice develop cardiac hypertrophy, while adiponectin overexpression reduces the hypertrophic response (549). The anti-hypertrophic actions of adiponectin can be attributed to modulation of the intracellular AMPK cascade. Cell culture experiments in cardiac myocytes show that adiponectin activates AMPK, and inhibits the hypertrophic response to α -adrenergic receptor stimulation (502).

Adiponectin's anti-hypertrophic effects can be reversed by transduction with dominant negative AMPK (156). Furthermore, in response to myocardial ischemia, adiponectin deficient mice display increased myocardial infarct size, myocyte apoptosis and myocardial TNF- α expression. On the other hand, overexpression of adiponectin reduces infarct size, apoptotic cell frequency and TNF- α levels (503). It appears that, in cultured myocytes, adiponectin's inhibition of apoptosis is mediated by its ability to activate AMPK signaling and that adiponectin also suppresses TNF- α production (501). In humans, it has been reported that elevated adiponectin levels are associated with lower risk of myocardial infarction in men and that adiponectin concentrations rapidly decline following myocardial infarction (277, 433). Moreover, lower levels of adiponectin are also associated with a further progression of left ventricular hypertrophy in patients presenting with hypertension, left ventricular diastolic dysfunction and hypertrophy (210).

Leptin

Leptin, predominantly produced by adipose tissue, was the first adipocyte hormone identified (76, 177, 186, 637). Leptin influences appetite and food intake through a direct effect on the hypothalamus (302). Leptin is also a marker of nutrition, as low plasma leptin concentrations serve as a "starvation signal", decreasing energy expenditure and stimulating the search for food in food deprived rodents (137, 220, 394). Leptin receptors have been identified in peripheral tissues including endothelial cells, platelets, monocytes/macrophages and the brainstem (337, 444, 633). In the obese state leptin is thought to contribute to insulin resistance and is considered one of the links between obesity, insulin resistance and atherosclerosis (37).

Plasma leptin concentrations are highly correlated with BMI (333). Mice deficient of the leptin coding gene, or *ob/ob* mice, are very obese and diabetic. If these

mice are treated with regular injections of leptin they reduce their food intake, their metabolic rate is increased and they lose weight (177, 186, 419). As animals and humans become obese, the role of leptin in regulating body weight becomes more complex. In most obese individuals leptin concentrations are high because of the increased amount of leptin secreting adipose tissue (84). There is also a suggested leptin resistance. It appears that as leptin concentrations rise, the hormone induces target cells to become resistant to its actions (360). As leptin concentrations increase, so does the expression of SOCS-3, a potent inhibitor of leptin signaling (360).

Leptin has important effects on peripheral metabolism as well. Leptin is able to reverse hyperglycemia in *ob/ob* mice before body weight is corrected (419). Furthermore, in a mouse model of congenital lipodystrophy resulting in insulin resistance, hyperinsulinemia, hyperglycemia, and fatty liver, leptin therapy reversed insulin resistance and diabetes (507). Leptin also improves glucose homeostasis in humans with lipodystrophy or congenital leptin deficiency (392). Administration of exogenous leptin to individuals with lipotrophic diabetes resulted in marked reductions in triglyceride concentrations, liver volume, and glycated hemoglobin and discontinuation or large reduction in anti-diabetes therapy (392). However, leptin failed to correct hyperglycemia in obese patients, further supporting the concept of “leptin resistance” in these individuals (203). Leptin improves insulin sensitivity in muscle by reducing intramyocellular lipid levels and activating AMPK (346). Leptin also enhances insulin sensitivity in the liver by decreasing intracellular hepatic triglyceride levels (249). Interestingly, exercise activates AMPK, which also increases fat oxidation and reduces insulin resistance (471). Thus, leptin, adiponectin and exercise may act via the same signal transduction pathway to increase fat oxidation and promote insulin sensitivity (177).

Leptin is also apparently involved in the pathogenesis of cardiovascular disorders. Hyperleptinemia is associated with atherosclerosis independent of insulin resistance (454). It has been shown that leptin deficient mice were protected from the development of atherosclerosis despite the contribution of all other metabolic factors that accelerate vascular disease (191). The mechanism of this is not completely understood, but the prolonged stimulation of the immune system seems to be one of the potential

mechanisms since leptin may enhance both proliferation and differentiation of hemopoietic cells, including T cells (322). Furthermore, leptin has multiple effects on cells of the arterial wall. In endothelial cells leptin induces oxidative stress, increases production of MCP-1 and endothelin-1, and potentiates proliferation (49, 411, 442, 614). In addition, leptin increases platelet aggregation and arterial thrombosis via a leptin receptor-dependent pathway, stimulates macrophage recruitment by inducing the release of monocyte colony-stimulating factor, promotes cholesterol accumulation in macrophages under high glucose conditions and stimulates angiogenesis (85, 278, 314, 377, 510). Leptin also promotes calcification of cells in the vascular wall and facilitates thrombosis by increasing platelet aggregation (363, 408). Finally, leptin appears to contribute to vascular disease via obesity-associated hypertension, not through metabolic actions, but via action on central sympathoregulatory pathways (88). Leptin increases peripheral sympathetic tone, and lower arterial pressures are found in leptin-deficient mice, thus suggesting a role for leptin in the development of hypertension (49, 442). These aforementioned effects of leptin may explain the epidemiological association between elevated levels and cardiovascular risk (37).

TNF- α

TNF- α was first described as an endotoxin-induced serum factor that causes necrosis of tumors (70, 372). Two TNF- α receptors, type 1 (TNFR1) and type II (TNFR2), mediate the TNF- α signal by forming protein complexes with cytoplasmic adaptor proteins (372). TNF- α plays a key role in the mediation of the immune response as a multi-functional regulator of inflammation, cell apoptosis and survival, cytotoxicity, and production of other cytokines, such as IL-1 and IL-6 (372). Obesity-associated TNF- α is primarily secreted from macrophages infiltrating adipose tissue, whereas the adipocytes predominantly produce un-secreted, membrane bound TNF- α (37, 587, 611). The secreted adipose tissue TNF- α is specifically increased in visceral adipose depots (558). TNF- α is thought to work mainly through autocrine or paracrine functions, where local concentrations would be more likely to exert its metabolic effects (222, 348, 629).

TNF- α was the first cytokine to be implicated in the pathogenesis of obesity and insulin resistance (215). Adipose tissue expression of TNF- α is increased in obese

rodents and humans and positively correlated with adiposity and insulin resistance (132, 215, 470). In obese humans, TNF- α expression is increased and improvements in this increased expression occur following weight loss (97, 263). Moreover, expression of TNF- α in obese *fa/fa* rats and *ob/ob* mice was increased and shown to regulate insulin action (215, 550). Mice lacking TNF- α or the TNF receptors had improved insulin sensitivity and decreased circulating fatty acids in both dietary and genetic models of obesity (567). At a molecular level, exposure of cells to TNF- α or elevated levels of free fatty acids stimulated inhibitory phosphorylation of serine residues on IRS-1, thus leading to insulin resistance (5, 417). Several potential mechanisms for TNF- α 's metabolic effects have been described. TNF- α activates serine kinases such as JNK and p38 mitogen-activated protein kinase (MAPK) that increase serine phosphorylation of IRS-1 and IRS-2, making them poor substrates for insulin receptor-activating kinases and increasing their degradation (214, 527).

TNF- α not only initiates, but also propagates atherosclerotic lesion formation (300). It is known that TNF- α activates the transcription factor nuclear factor- κ B (NF- κ B), which accelerates experimental atherogenesis, in part by inducing the expression of VCAM-1, ICAM-1, MCP-1, and E-selectin in aortic endothelial and vascular smooth muscle cells (395). This results in inflammatory and foam cell accumulation (38). Moreover, TNF- α reduces NO bioavailability in endothelial cells and impairs endothelium-dependent vasodilation, promoting endothelial dysfunction (38, 581). Despite these intriguing in vitro data, animal studies focused on TNF- α and the development of atherosclerosis have produced mixed results (37). Reducing TNF- α concentrations in apoE deficient mice resulted in a significant decrease in atheromatous lesions, however, in wild-type mice reduced TNF- α levels produced no improvements (51, 490). In addition, mice deficient of the p55 TNF- α receptor exhibited accelerated atherosclerosis (489). Finally, although TNF- α is thought to play a role in the progression of ischemia-related congestive heart failure, anti- TNF- α therapy has produced no benefits for congestive heart failure progression (265). Despite these conflicting data, TNF- α still may very well partake in the pathogenesis of atherosclerosis and vascular disease.

Interleukin-6

IL-6 belongs to a family of cytokines that activate target genes involved in cell differentiation, survival, apoptosis and proliferation (76). IL-6 is one of the primary pro-inflammatory mediators secreted by immune system cells and is a key player in haematopoiesis and acute phase and immune responses (198). IL-6 and its soluble receptor activate endothelial cell production of chemokines and upregulate expression of adhesion molecules resulting in the recruitment of leukocytes to inflammatory sites, and may well regulate TNF- α and IL-1 synthesis through autocrine and paracrine loops (463, 531). IL-6 binds to a plasma membrane receptor complex containing the common signal transducing receptor chain gp 130. Signal transduction involves the activation of JAK tyrosine kinase family members, leading to activation of transcription factors from the STAT family. IL-6 also appears to signal through the MAPK cascade (198).

Circulating IL-6 concentrations are positively correlated with obesity, impaired glucose tolerance and insulin resistance (31). Several studies have reported the positive relationship between body mass index and plasma IL-6 concentrations (328, 409). 20-30 % of this cytokine in circulation is produced by adipose tissue. In obese subjects, with elevated waist-to-hip ratios, this participation is even greater (348). It has been documented that visceral adipose tissue releases 2 to 3 times more IL-6 than does subcutaneous adipose tissue (138, 152). This cytokine may also be involved in the pathogenesis of insulin resistance, as insulin sensitivity is enhanced in diet-induced obese mice treated with anti-IL-6 antibodies (275). In addition, plasma IL-6 concentrations predict the development of type 2 diabetes mellitus (575). Fasting plasma IL-6 levels were negatively correlated with the rate of insulin-stimulated glucose disposal in Pima Indians (222, 575). Peripheral administration of IL-6 induces hyperlipidemia, hyperglycemia, and insulin resistance in rodents and humans (428, 529, 559). IL-6 impairs insulin signaling through a down-regulation of IRS and an up-regulation of SOCS-3 (457). Bastard and colleagues (31) have reported that IL-6 concentrations are more strongly correlated with obesity and insulin resistance than TNF- α , and that very low-calorie diet results in significant decreases in circulating IL-6 concentrations in obese women. A variety of other studies also demonstrate that weight loss results in decreased levels of circulating IL-6 (120, 167, 279). Moreover, circulating IL-6 stimulates the

hypothalamic-pituitary-adrenal axis, which is associated with central obesity, hypertension and insulin resistance (628).

Obesity associated induction of adipose IL-6 production causes the secretion of hepatic acute phase response proteins including CRP, fibrinogen, serum amyloid-A and α -1 antichymotrypsin (642). It is well known that CRP is both a marker of and an important risk factor for cardiac events and atherosclerosis in the general population (61, 76). Recent data suggest that CRP might directly elicit endothelial dysfunction and atherogenesis at the vessel wall (107, 414, 512). Furthermore, IL-6 stimulates the production of fibrinogen and platelet activity, which increase the risk of clot formation (62). There are data that suggest IL-6 decreases lipoprotein lipase activity, which results in elevated circulating FFA and macrophage uptake of lipids. In young atheromatous lesions, macrophage foam cells and smooth muscle cells express IL-6, suggesting a role for this cytokine in the early stages of atherosclerosis (628). These data suggest a role for the cytokine IL-6 in the pathogenesis of obesity related metabolic and cardiovascular disorders.

Summary

It is recognized that adipose tissue functions as both an energy storage and secretory tissue producing a variety of bioactive peptides. Adipocytes from lean, healthy individuals secrete cytokines that coordinate systemic metabolic processes and protect cardiovascular tissues from stress (578). Moreover, complex interactions exist between cytokines, inflammation and the adaptive responses in maintaining homeostasis and health (115). Obesity causes an imbalance in cytokine secretion (578). Abnormalities in cytokines, their receptors and the signaling pathways that they initiate such as IKK β , JNK and JAK/STAT are involved in a wide variety of diseases (130). The cytokines, and possibly more appropriately adipokines or adipocytokines, that may provide a molecular link between obesity and the development of MetS, type 2 diabetes and cardiovascular disease are of particular interest. These cytokines include well-characterized bioactive peptides such as adiponectin, TNF- α and IL-6, as well as a wide variety of less understood cytokines including cardiotrophin-1 and fibroblast growth factor-21.

Weight Loss and Lifestyle Modification

Weight loss through lifestyle modification is beneficial for treating excessive adiposity, dyslipidemia, hypertension, hyperglycemia and insulin resistance among other metabolic disorders (87, 413). The magnitude of weight loss does not need to be drastic, as even modest weight loss of 5-10% significantly attenuates metabolic dysfunction (87). The Finnish Diabetes Prevention Study demonstrated that lifestyle intervention with modest weight loss significantly reduced the prevalence of MetS (221). Furthermore, the intensive lifestyle intervention with moderate weight loss utilized in the Diabetes Prevention Program also resulted in a 41% reduction in the incidence of MetS (393).

Current therapies that cause weight loss by inducing a negative energy balance include dietary intervention, physical activity, pharmacotherapy and surgery. Behavior modification to enhance dietary and activity compliance is an important component of all these treatments. At present, the therapeutic intervention used does not appear to be relevant to the benefit of weight reduction with a few exceptions (434). However, the use of caloric restriction through dietary modification and enhanced energy expenditure through physical activity are the most commonly prescribed therapies to induce modest weight loss.

A decrease in caloric intake is an avenue by which to create a negative energy balance resulting in weight loss (476). It is prudent to recommend a reduced calorie diet low in saturated fat, higher in unsaturated fat, high in whole grains and low in sodium (87). However, more important than the composition of the diet, is the overall caloric intake. Regardless of which macronutrients are emphasized, hypo-caloric diets are key to inducing meaningful weight loss (476).

A lifestyle aimed at increasing physical activity/energy expenditure and decreasing, or even maintaining, body weight is another important approach to reducing health risks (87). Increased physical activity and higher cardio-respiratory fitness, independent of weight loss, are associated with decreased CVD risk and lower incidence of type 2 diabetes and MetS (298, 383, 585, 622). Exercise is also particularly effective at improving insulin sensitivity as well as reducing dyslipidemia and hypertension (23, 106, 474, 524). Cardiorespiratory fitness seems to have an independent effect on metabolic function in some aspects; however, a change in body weight and body

composition (particularly abdominal adiposity), is an important mediator in the ability of increased physical activity to modify chronic disease risk (87). Regular exercise appears to play an important role in abdominal fat loss and the metabolic changes that ensue (466).

Inflammation and Weight Loss

Weight loss and a reduction in adipose tissue mass are associated with a decrease in obesity related markers of inflammation regardless of whether weight loss is induced by caloric restriction (20, 80, 259, 462, 613), exercise (387, 462) or a combination of the two (462). However, when employing dietary restriction, studies using low-calorie diets as the method of intervention resulted in the highest mean weight loss, compared with those using other methods such as low-fat diets. The greatest improvements in circulating concentrations of obesity related markers of inflammation were observed in studies reporting at least 10% weight loss (47, 59, 84, 426, 447, 545). For example, Kasim-Karakas and colleagues failed to show any significant changes in serum CRP, IL-6 or adiponectin with 8% (6 kg) weight loss after a 12-month low-fat diet (258). On the other hand, two low-calorie diet investigations reported a weight loss of approximately 15% with a 32% decrease in plasma CRP after 14 months and a 24% reduction in plasma IL-6 after only 6 months (59, 84). Moreover, You and colleagues showed that a 5% (5 kg) weight reduction was unable to induce significant improvements in markers of inflammation (625). Bougoulia et al. demonstrated a 22, 21, 86, and 32% decrease in plasma CRP, TNF- α , IL-6, and leptin, respectively, and a 72% increase in plasma adiponectin concentrations with a 19% (20 kg) weight loss (47). In addition, Hannum et al. failed to show a significant decrease in serum CRP with modest weight loss (4-7% or 4-6 kg) in either a self-selected or portion-controlled low-calorie, low-fat diet (190). Therefore, based on this data, the benefits of weight loss greater than 10% can clearly be seen (142). Yet, this does not discount the positive benefits associated with a modest 5-10% weight loss. For example, Heald and colleagues reported that a reduced-fat diet resulted in a small but significant weight loss of 2 % (1 kg) and a significant 24% decrease in serum CRP (197).

Few studies have explored the effect of weight loss on inflammatory markers through increased physical activity alone (387, 462, 530, 560). In these studies, the resulting changes in obesity related markers of inflammation are fairly inconsistent. For example, TNF- α significantly decreased by 16% after 12 weeks in one investigation (530), decreased by 83% after 5 months in another (560), and significantly increased by 12% in a third intervention (462). These differences are not explained by changes in body composition or the degree of weight loss. However, differing intensities and/or frequencies of exercise training in each investigation, or possibly the duration of the intervention may explain the observed findings (142).

Interventions combining both diet and physical activity modifications also report significant weight loss and improvements in obesity related markers of inflammation (55, 78, 97, 114, 119, 120, 335, 371, 379, 462, 473, 498, 519, 625, 632, 639). For example, Ryan and colleagues (473) observed reductions in plasma CRP and IL-6 of 7 and 16%, respectively with modest weight loss. Moreover, with 7-9% weight loss You et al. (625) found that plasma CRP, IL-6 and TNF- α concentrations decreased by 34, 27 and 6% respectively. The three investigations that resulted in the greatest amount of weight loss were also the longest in duration (1 – 2 years) (97, 120, 335). Significant improvements in inflammatory markers were also reported. The decrease in serum TNF- α concentrations ranged from 24 – 31% (97, 335), the decrease in serum CRP ranged from 34 – 44% and the decrease in serum IL-6 ranged from 33 – 67 % (120, 335). Serum adiponectin concentrations were shown to increase by 48% (97). These three studies were performed in women, however similar improvements in CRP and IL-6 were also found in men (119). These studies clearly demonstrate the benefit of weight loss through dietary and exercise modification in ameliorating obesity-induced inflammation (142).

Insulin Resistance, Diabetes and Weight Loss

A variety of studies have shown that modest weight loss through caloric restriction (20, 80, 361, 613), increased physical activity and a combination of both (315, 410, 452, 582, 588) serves to improve insulin sensitivity and ameliorate insulin resistance. Weight loss has a dramatic effect on blood glucose values in individuals with or without diabetes. It is estimated that for every 1 kg of weight loss, plasma glucose

decreases by roughly 0.2 mM. Thus, a modest 5 kg weight loss would decrease average fasting plasma glucose by 1 mM or 18 mg/dL. This improvement is in the range that is provided by many of the oral hypoglycemic agents that are commonly approved by the FDA (9). The US Diabetes Prevention Program Research Group recently reported data on a large randomized clinical trial of type 2 diabetes prevention (276). Over 3200 overweight adults with impaired glucose tolerance were included in the study. The intensive lifestyle intervention group received diet, exercise and behaviour modification counseling, and aimed for a 7% reduction in body weight. Average weight loss was roughly 4 kg at the end of the follow-up period. This lifestyle intervention and subsequent weight loss was highly effective in preventing/delaying the appearance of type 2 diabetes. Compared with the placebo/control group, there was a 58% reduction in the incidence of diabetes in the lifestyle intervention group (276, 569). Furthermore, The DPP showed that weight loss was the number one predictor of reduction in the incidence of diabetes. In fact, for every kilogram of weight loss, the risk of diabetes development was decreased by 16% (189).

The Finnish Diabetes Prevention Study (FDPS) also reported that modest weight reduction could limit the incidence of type 2 diabetes (563). This study included subjects with impaired glucose tolerance divided into a lifestyle intervention group and a control group. After 1 year the average weight loss in the intervention group was 4 kg (5% weight reduction), resulting in a 58% lower cumulative incidence of diabetes as compared to subjects in the control group. This weight loss further beneficial in reducing waist circumference, plasma triglycerides and systolic and diastolic blood pressure, emphasizing the pleiotropic effects of modest weight loss (563, 569).

Cardiovascular Disease Risk Factors and Weight Loss

Weight loss systematically reduces risk factors for cardiovascular disease (8). A number of studies demonstrate that modest weight loss achieved by caloric restriction (20, 80, 259, 361, 435, 613), exercise and combination of both (410, 570) is effective in normalizing cardiovascular disease risk factors such as hypertension and dyslipidemia. For example, Dengel and colleagues (105) studied 12 overweight individuals with known diabetes or vascular disease. During weight loss, there were significant reductions in

BMI and body fat percentage along with improvements in total cholesterol, LDL-C, triglycerides and insulin sensitivity. After 6 months, there were also significant improvements in brachial artery compliance and distensibility (105). According to a meta-analysis conducted by Dattilo and Kris-Etherton (99), a weight loss of just 1 kg reduces serum cholesterol values by 2.28 mg/dL, LDL-C by 0.91 mg/dL, and triglycerides by 1.54 mg/dL, and increases serum HDL-C by 0.07 mg/dL. Furthermore, Balkestein et al. (26) reported that 3 months of weight loss induced by negative caloric balance improved carotid distensibility in obese men. Thus, along with improvements in blood lipids, weight loss may also lead to enhanced vascular function.

Moreover, blood pressure seems to decrease in a linear manner with weight loss (8). According to a meta-analysis from MacMahon et al. a small 1 kg weight loss results in an average systolic blood pressure reduction of 0.49% or 0.68 mmHg, whereas diastolic blood pressure decreases 0.38% or 0.34 mmHg. This same report observed a 6.3 mmHg reduction in systolic blood pressure and a 3.1 mmHg reduction in diastolic blood pressure with a 9.2 kg weight loss (329). Anderson and colleagues found that with a greater weight loss of, on average, 35.3 kg in morbidly obese subjects, the reductions of systolic and diastolic blood pressure were 13 mmHg and 9.6 mmHg, respectively (8). This equates to a reduction in blood pressure of roughly 10% (9). However, it should be noted that only about 85% of hypertensive obese individuals have significant reductions in blood pressure with significant weight loss, indicating that other factors are at play a role in regulating hypertension in obese individuals (8).

Furthermore, the US Trials of Hypertension Prevention, Phase II evaluated the impact of weight loss on the incidence of hypertension (528). It included 1,191 overweight individuals with borderline hypertension (systolic <140 mmHg, diastolic 83-89 mmHg). These subjects were allocated to either a control group or a weight loss intervention group aiming at a 4.5 kg weight reduction during the first 6 months of the trial. This weight loss was to be maintained until the 36 months period. The weight loss intervention included behavior modification consisting of dietary change and increased physical activity. The control group experienced a slight weight gain during the study period. The intervention group had a mean weight reduction of 4.4 kg at 6 months, 2.0 kg and 18 months and 0.2 kg at 36 months. Although the initial weight loss was not

maintained, mean body weight in the intervention group was always significantly less than that of the control group. At the 6, 18 and 36 month follow-up periods, the cumulative incidence of hypertension was significantly lower in the intervention group. There was a dose response relationship between the degree of weight loss and the amount of blood pressure reduction. Subjects who lost 4.5 kg or more at 6 months and maintained that weight loss over 36 months demonstrated a 65% reduction in hypertension risk as compared to those who did not lose weight (528, 569).

Oxidative Stress and Weight Loss

Weight loss and fat loss through dietary restriction and/or increased physical activity appears to have positive effects on oxidative stress as well (573). A 4-week dietary weight loss study in 9 obese patients measured oxidative stress before and after the weight loss program. All subjects were restricted to a 1000 kcal/day diet. Weight loss (4.5 +/- 2.8 kg) was accompanied by a 13% reduction in plasma TBARS (a biomarker of oxidative stress) and a significant reduction in ROS. Reductions in all oxidative stress biomarkers occurred rapidly in obese subjects after only one week of dietary restriction. This benefit persisted until week 4, but disappeared after the cessation of treatment (96). Chen and colleagues examined the relationship between dietary patterns and oxidative stress. 122 premenopausal women were randomized into one of four groups for a 12-month program. The groups included: no intervention, low-fat (15% energy from fat), high fruit and vegetable intake (nine servings per day), and combined low-fat, high fruit and vegetable intake group. Following the intervention, weight loss occurred only in the low-fat groups. Concentrations of 8-isoprostane were also only reduced in the low-fat groups. Moreover, changes in BMI were directly correlated with changes in 8-isoprostane following the intervention. Therefore, the authors concluded that weight loss directly resulted in reductions in oxidative stress (72). Controlled food intake also reduces blood insulin and glucose concentrations, thereby suppressing insulin-induced free radical formation that may occur in obesity (297). Thus, caloric restriction and diet modification may reduce body weight and free radical/ROS formation in obese individuals, both of which lower oxidative stress (573).

A 3-week combined diet and exercise intervention consisting of high carbohydrate/low fat diets and 1.5 hours of daily exercise was administered to 80 obese individuals. Following the program, body weight was reduced by 4-5% and LDL shifted from small pro-atherogenic to large less-atherogenic particles. Furthermore, the basal level of serum lipid oxidation was reduced by 21%. There was no change in LDL vitamin E content, but LDL β -carotene content increased by 46% (32). Therefore, diet and exercise intervention shifted the LDL particles to the less atherogenic large particle size and reduced oxidative stress in obese individuals (573). In a study carried out by Roberts and colleagues, obese men completed a 3-week short-term Pritikin vegetarian diet regimen and walked for 45-60 min/day. Average weight loss was 4 kg or 3.8% of body weight. This weight loss resulted in reduced total cholesterol, LDL-C and triglycerides and increased HDL-C. Serum 8-isoprostane levels were also reduced from 210 to 150 pg/ml from baseline to post-treatment (458). Again, rapid reductions in oxidative stress were observed with short-term weight loss. These reductions may be associated with improvements in blood lipids that accompany dietary change and weight loss (573).

One recent study compared the effect of adding exercise to Orlistat treatment in ameliorating oxidative stress (403). Participants were placed into either an Orlistat treatment group or an Orlistat-exercise group (cycle ergometer, 3 days/week, 45 min/session). Following the 12-week intervention weight loss was 8.5% for the Orlistat group and 10.2% for the combined treatment group. MDA (a marker of oxidative stress) levels were 29.9% lower in the Orlistat-exercise group as compared to the Orlistat-only intervention. Both groups showed reductions in serum vitamin A and E levels; however, these values remained higher in the Orlistat-exercise group. Therefore, the authors concluded that the combination of weight loss and exercise-induced up-regulation of antioxidant defenses in the combined treatment group were effective in lowering systemic oxidative stress (403, 573).

In addition, animal studies provide evidence regarding exercise, weight loss and the reduction of oxidative stress. Saengsirisuwan et al. (477) separated obese Zucker rats into a control group, lipoic acid group (30 mg/kg body weight), exercise group (treadmill running for 60-75 min/day) and lipoic acid and exercise group. Following the 6-week intervention, all treatment groups had lower body weights than the control

animals. Oxidative stress levels were significantly lower in all treatment groups and this was associated with improvements in peripheral glucose uptake (477). Based on this and previous data, exercise-induced weight loss appears to be effective in reducing oxidative stress.

Temporal Effects of Weight Loss and Significance of Study

Modest weight loss of roughly 5 – 10% of initial body weight has been demonstrated to have beneficial effects on metabolic and cardiovascular risk factors associated with obesity. Hypo-caloric diets and regularly practiced exercise can create the negative energy balance necessary to generate these beneficial effects. However, the temporal effects of weight loss are not yet firmly established in the literature. Evidence suggests that metabolic improvements may occur rapidly within the first few weeks of lifestyle modification and continue at a slower rate as weight loss continues. Clinical markers of metabolic health that demonstrate this trend include triglycerides, glucose, insulin, hemoglobin A1c, insulin sensitivity and biomarkers of oxidative stress (96, 262, 318, 345, 478, 513).

It is possible that alterations in circulating levels of CT-1 and FGF-21 may mirror these changes based on mechanisms and relationships presented in the literature. For example, CT-1 concentrations are elevated along with increased levels of insulin and glucose (365). CT-1 also down-regulates the nuclear receptor PPAR- γ most likely resulting in decreased expression of the PPAR- γ target and insulin sensitizing agent adiponectin (358, 645). Furthermore, CT-1 and reactive oxygen species appear to regulate one another through a positive feedback loop in that CT-1 potentiates ROS production, which in turn, leads to further CT-1 expression (21, 482).

In obese humans, FGF-21 concentrations are elevated along with circulating fatty acids, glucose and insulin (73, 111, 308, 636). FGF-21 is also a gene target of PPAR- γ , which up-regulates adiponectin expression and inhibits the formation of ROS (357, 358, 580). Weight loss results in an up-regulation of PPAR- γ that should cause an increase in FGF-21 and adiponectin and a decrease in ROS (568). However, FGF-21 levels are already increased in states of human obesity suggesting a possible FGF-21 resistance (73, 308, 475, 636). Based on this data, FGF-21 levels may decrease with weight loss, despite

being a gene target of PPAR- γ . In other words, reductions in FGF-21 hypothesized here to occur with weight loss will likely occur along with elevations in adiponectin and decreases in biomarkers of oxidative stress. It is possible that the link between FGF-21 and levels of fatty acids, glucose and insulin may be stronger than the effects levied by weight loss-induced up-regulation of PPAR- γ . Based on these specific relationships, CT-1 and FGF-21 concentrations should decrease along with weight loss-induced reductions in glucose, insulin, NEFAs and biomarkers of oxidative stress and elevations in adiponectin demonstrated in the literature (96, 318, 373, 403, 412, 413, 439, 513, 568).

Recent evidence supports the theoretical role of cytokines and adipokines in obesity and weight loss (37, 126, 143, 222). Both CT-1 and FGF-21 may be related to the metabolic dysfunction occurring with obesity as well as play a role in the metabolic and cardiovascular health improvements associated with weight loss (268, 365, 636, 645). However, there appears to be limited data in the literature that directly addresses the response of CT-1 and FGF-21 to weight loss in humans. Therefore, the importance of this study lies in its potential to contribute foundational clinical information that will be useful and relevant to our understanding of the responses of CT-1 and FGF-21 to obesity, glucose metabolism and modest weight loss.

Chapter III.

Methods

Subjects

This study used a sample cohort of subjects initially recruited for participation in a larger investigation. We analyzed the data from 9 obese participants that completed a weight loss program and 7 aged matched controls. All volunteers met the following characteristics in order to be eligible for the weight loss portion of the study: 1) male between 30 to 65 years of age; 2) body mass index (BMI) was greater than 30 kg/m^2 and/or body fat was greater than 30% of total body weight and waist girth was greater than 40 inches; 3) weight stable over previous 6 months; 4) without previously diagnosed cardiovascular or metabolic disease; 5) exhibited no signs or symptoms of latent heart disease; 6) no regular exercise over the past six months and did not have a job requiring strenuous physical activity; 7) non-smoker; 8) did not take medication known to alter lipid or glucose metabolism, and; 9) did not exhibit conditions that would prevent regular treadmill walking. Control subjects also exhibited each of these characteristics excluding number 2. These individuals had a BMI below 27 kg/m^2 .

Preliminary Screening and Baseline Procedures

Volunteers were recruited by flyers placed around the Auburn-Opelika area, as well as through Auburn University's daily faculty/staff announcement e-mail. Volunteers were scheduled to visit the lab in order to undergo preliminary screening procedures. Initially, volunteers were given a health history questionnaire and physical activity questionnaire to complete. Subsequently, height, weight, waist girth and resting blood pressure were measured. If all criteria for inclusion in the study were met, individuals were scheduled to return to the lab and underwent a physical exam by a physician. Following the physical exam, a blood sample was obtained via venipuncture

from an antecubital vein and conducted a body composition assessment using total body x-ray (DEXA) scan to determine body fat levels and fat distribution patterns. Volunteers were then asked to perform a maximal exercise test (GXT), using the standard Bruce protocol, on a motor-driven treadmill to determine cardiovascular fitness (301). For this test, a 12-lead electrocardiogram was administered at rest and throughout the exercise test. Blood pressures were obtained periodically throughout the GXT to further determine cardiovascular responses to exercise. During the GXT, respiratory gases were measured by a Medical Graphics Ultima metabolic unit (MedGraphics, St. Paul, MN). Furthermore, heart rate and ratings of perceived exertion were monitored throughout the test.

Following the preliminary screening procedures, volunteers who met the necessary criteria were asked to report to the lab after two days of stable diet and physical activity and after a 10 to 12 hr overnight fast. After obtaining body weight and blood pressure, a blood sample was collected via venipuncture in an antecubital vein. This fasting blood sample was used as the baseline sample for all biochemical variables. Participants also recorded their diet and physical activity for 7 days (starting 2 days before lab visit for fasting blood sample) for the purpose of baseline assessment.

Weight Loss Procedures

Volunteers were randomly assigned to either an exercise or dietary restriction intervention in order to achieve an 8 to 10% weight loss, as recommended by the National Heart, Lung and Blood Institute (NHLBI) (1). The exercise and diet interventions were initially designed to achieve a 2000 to 2500 kcal/week energy expenditure or energy deficit determined from our initial energy requirement estimates. The overall targeted weight loss for both interventions was 8 to 10% of initial body weight over a 6 to 10 month period (1). Initially, both groups underwent nutritional counseling and education regarding how to use the American Dietetic Association Food Exchange Program (2). The food exchange program was used to help participants maintain specific energy intake recommendations over the course of the weight loss period.

Every 4 weeks each participant scheduled an appointment where a fasting blood sample was collected , DEXA scan performed, and current dietary and nutrient intake and exercise prescription was reviewed and modified. Also, subjects turned in 3-day diet and physical activity record. Recommendations at the monthly meeting were based on the information provided in exercise logs (exercise intervention only), 3-day diet records and the rate of weight loss achieved over the previous month. Modifications to an intervention were based on adaptations in metabolic efficiency known to occur with caloric restriction and increased energy expenditure (341). Once participants achieved the targeted weight loss, we administered an OGTT and collected a 3-day diet and physical activity log (starting 2 days before OGTT) for the purpose of post-weight loss assessment.

Exercise Intervention

If assigned to the exercise intervention, participants achieved their weekly energy expenditure goal by exercising 4 to 5 times per week. Each individual was asked to complete two exercise sessions per week in the lab under direct supervision. The in lab exercise sessions included monitoring exercise intensity, obtaining a body weight and blood pressure and reviewing outside physical activity records/exercise logs. These exercise sessions were monitored (respiratory gases and heart rates measured) and modified to achieve the desired work rates and corresponding heart rate range to meet the exercise session energy expenditure goal (400 to 500 kcal/session). Each participant was trained and counseled in the use of a heart rate monitor and rating of perceived exertion (RPE) scale for monitoring exercise intensity. Further training included record keeping for maintaining an exercise log. Apart from the two exercise sessions per week under laboratory supervision, participants completed the remaining exercise sessions on their own in order to achieve the recommended weekly caloric expenditure.

Diet Intervention

If assigned to the diet intervention, participants achieved their weekly energy deficit goal by initially reducing daily caloric intake 300 to 350 kcal/day. The general counseling strategy for reducing daily caloric intake was to reduce portion sizes and to

replace calorically dense foods and those high in saturated fats with less-dense alternatives containing poly- and monounsaturated fats (87, 476). Subjects visited the lab two times per week for body weight and blood pressure measurement and review of their outside physical activity record. Any dietary issues or questions that subjects may have had were resolved during these regular visits.

Biochemical Procedures and Analysis

Fasting blood samples were collected into one 10 ml “red top” serum vacutainer tube and one 4 ml “purple top” potassium EDTA tube (Becton Dickinson Vacutainer, Franklin Lakes, NJ). Blood samples for each time point during the OGTT were collected into one 10 ml “red top” serum tube. Hemoglobin and hematocrit levels were measured from each blood sample collected in the potassium EDTA tubes. The remaining blood in all tubes was allowed to clot for 30 minutes and then centrifuged for 10 minutes to separate the serum and plasma. Aliquots of both serum and plasma were transferred into 2 ml ultracentrifuge tubes and subsequently stored at -70 degrees centigrade for future analysis.

FGF-21 (Millipore, St. Charles, MO), total adiponectin (Alpco Diagnostics, Salem, NH), TNF- α (R&D Systems, Minneapolis, MN), IL-6 (R&D Systems, Minneapolis, MN), and myeloperoxidase (MPO) (Alpco Diagnostics, Salem, NH) were determined by enzyme-linked immunosorbent assay (ELISA). Total antioxidant capacity (TAC) (BioVision Inc., Mountain View, CA), non-esterified fatty acids (NEFA) (Wako Diagnostics, Richmond, VA), and glucose (Wako Diagnostics, Richmond, VA) concentrations were determined by an enzymatic colorimetric assay. CT-1 (Christchurch Cardio-Endocrine Research Group, Christchurch, NZ) (421) and insulin (Millipore, Billerica, MA) were determined by radioimmunoassay (RIA). The homeostasis model assessment (HOMA) using fasting insulin and glucose concentrations was used to estimate insulin resistance as follows: [fasting insulin μ U/mL x fasting glucose (mmol/L)]/22.5. The quantitative insulin sensitivity check index (QUICKI) was calculated as follows: $1 / (\log (\text{fasting insulin } \mu\text{U/mL}) + \log (\text{fasting glucose mg/dL}))$.

Statistical Analysis

For the purpose of statistical analysis the data from both the diet and exercise groups were combined due to no observable difference in the extent or pattern of weight loss between groups. The primary dependent variables of interest were plasma concentrations of CT-1 and serum concentrations of FGF-21. We measured myeloperoxidase and total antioxidant capacity (TAC) as biomarkers of oxidative stress. Cytokines measured were total adiponectin, TNF- α , and IL-6. Clinical markers of metabolic health that served as dependent variables were non-esterified fatty acids (NEFA), glucose and insulin. Glucose to insulin ratio, HOMA and QUICKI were indexes used as surrogate markers of insulin resistance and insulin sensitivity. We used multiple statistical procedures to analyze our data. In order to address question 1, an independent t-test was employed to determine differences between obese and lean participants. A paired t-test was used to analyze changes that occurred in the obese group with 8 – 10% weight loss. Temporal changes with weight loss were analyzed using 1 x 6-10 repeated measures ANOVAs. Duncan's New Multiple Range test was employed to describe significant differences determined from ANOVAs. In order to examine the temporal response, weight loss was captured at each month and partitioned into percentages as follows: 2 – 4 %, 4 – 6 %, 6 – 8 % and target/post-weight loss. The lowest monthly weight achieved and the corresponding variables from DEXA scans and fasting blood samples that fell within each range were used for statistical analysis. Pearson product moment correlation coefficients were used to determine the relationship between baseline physiological characteristics and dependent variables in the obese and lean groups as well as changes observed with weight loss in the obese group.

Chapter IV.

Results

Baseline Physiological Characteristics

The data from nine obese participants and seven age-matched controls are reported.

Table 4a. Baseline physiological characteristics

Variable	Obese n = 9			Control n = 7		
	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max
Age	41.5 \pm 7.1	30	54	42.3 \pm 8.5	31	53
Height (in)	68.9 \pm 3.6	64.5	74.0	68.3 \pm 3.0	64.0	73.0
Weight (kg)*	101.7 \pm 21.0	81.6	137.9	74.5 \pm 5.0	64.5	78.6
BMI*	32.8 \pm 3.6	30.1	39.2	24.8 \pm 1.4	22.9	26.9
Waist girth (in)*	43.4 \pm 4.7	38.0	52.0	34.9 \pm 2.5	30.5	37.5
Total fat mass (kg)*	34.4 \pm 9.2	24.8	55.7	18.2 \pm 4.3	9.0	21.7
% fat*	35.2 \pm 4.3	29.5	43.5	25.2 \pm 5.5	14.4	31.4
Android fat mass (kg)*	8.6 \pm 2.3	6.0	12.5	5.7 \pm 0.8	4.2	6.6
% android fat*	47.4 \pm 5.2	39.9	55.7	35.2 \pm 9.3	16.4	43.8
Gynoid fat mass (kg)*	14.8 \pm 3.0	11.4	20.3	11.3 \pm 0.9	9.7	12.7
% gynoid fat*	36.0 \pm 5.1	29.5	44.8	28.9 \pm 5.9	21.0	38.5
Lean mass (kg)	62.8 \pm 12.3	47.7	88.6	53.6 \pm 3.6	47.5	59.2
% lean*	64.8 \pm 4.3	56.5	70.5	74.8 \pm 5.5	68.6	85.6
VO _{2max} (L/min)	3.03 \pm 0.39	2.43	3.60	2.59 \pm 0.47	1.82	3.08
VO _{2max} (mL/kg/min)	30.4 \pm 3.8	24.9	37.4	35.2 \pm 7.3	25.6	47.9
SBP (mmHg)*	131 \pm 6	124	144	118 \pm 12	104	142
DBP (mmHg)	82 \pm 7	68	90	76 \pm 6	70	88

Table 4b. Baseline humoral and metabolic parameters

Variable	Obese n = 9			Control n = 7		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
TC (mg/dL)	196 ± 30	154	238	191 ± 31	147	230
TG (mg/dL)	202 ± 102	74	344	157 ± 66	68	230
HDL-C (mg/dL)	36 ± 6	26	46	43 ± 7	35	52
LDL-C (mg/dL)	119 ± 27	82	159	116 ± 29	81	168
NEFA (mEq/L)*	0.70 ± 0.25	0.44	1.22	0.37 ± 0.20	0.13	0.69
Glucose (mg/dL)	91 ± 11	76	107	95 ± 5	87	104
Insulin (μU/mL)*	24.3 ± 9.9	17.0	46.5	7.5 ± 7.1	2.5	20.9
GIR*	4.09 ± 1.05	2.26	5.25	22.29 ± 13.85	4.55	41.60
HOMA*	5.60 ± 2.68	3.64	12.06	1.75 ± 1.65	0.64	4.90
QUICKI*	0.302 ± 0.015	0.271	0.316	0.373 ± 0.044	0.303	0.414
CT-1 (nmol/L)	0.295 ± 0.087	0.143	0.414	0.239 ± 0.099	0.049	0.382
FGF-21 (pg/mL)	433 ± 152	265	714	408 ± 219	72	727
Adiponectin (ng/mL)*	4047 ± 1712	1705	6315	2862 ± 788	1581	4064
TNF-α (pg/mL)	1.35 ± 0.34	1.08	2.13	1.04 ± 0.45	0.28	1.53
IL-6 (pg/mL)	1.98 ± 0.94	1.09	3.80	1.73 ± 1.39	0.72	4.62

All values presented as means ± standard deviation along with minimum and maximum values in range. * indicates a significant difference between groups ($p < 0.05$). Total a regional tissue mass is expressed absent bone mineral content. VO_{2max} = the maximal oxygen consumption measured over one minute of a standard Bruce protocol graded exercise test; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; TG = triglycerides; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; GIR = glucose to insulin ratio; HOMA = homeostasis model score; QUICKI = quantitative insulin sensitivity check index; MPO = myeloperoxidase; TAC = total antioxidant capacity.

All obese participants were caucasian. Five of the nine obese individuals were classified as having metabolic syndrome as described by NCEP ATP III (182). Eight subjects qualified for the MetS parameter of waist girth above 40 inches. Three participants had

blood glucose levels above 100 mg/dl, five exhibited triglyceride levels above 250 mg/dl and six had HDL-C concentrations below 40 mg/dl.

Control participants included in the study were sedentary, otherwise healthy individuals that maintained a BMI of less than 27 kg/m². Four individuals in the control group were caucasian, one was asian, one was black and one was of Latin American descent. The baseline physiological and humoral characteristics of the study participants are provided above in Tables 4a and 4b, respectively.

By design body weight, total and regional body fat, BMI and waist girth differed significantly between groups ($p < 0.05$). Humorally, obese and age-matched control individuals did not differ significantly in a majority of the variables measured at baseline ($p > 0.05$). However, NEFA and insulin concentrations and indexes of insulin sensitivity (GIR, HOMA, QUICKI) were significantly different between groups, indicating greater insulin sensitivity in control individuals ($p < 0.05$). At baseline the obese group had significantly higher adiponectin levels when compared to controls ($p < 0.05$)

Weight Loss and Tissue Loss

Obese participants lost an average of 19.7 ± 4.2 lbs or roughly 8.9% of initial body weight. The weight loss ranged from 12.8 lbs to 27.9 lbs or from 7.6% to 10.5%. On average, target weight loss occurred at 6.3 ± 1.8 months. Percent changes in body weight are provided in Figure 5.

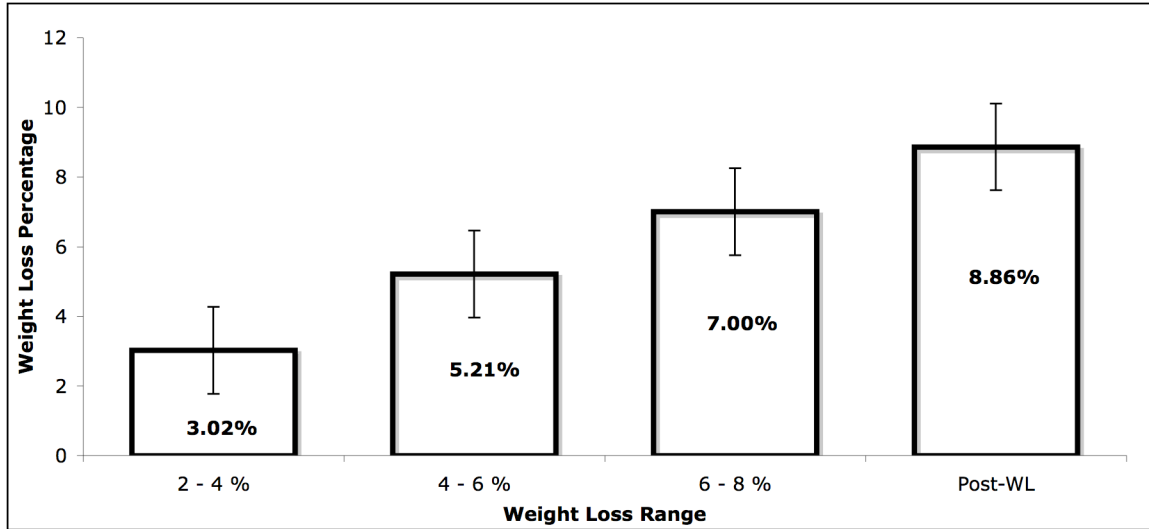


Figure 5. Percent changes in body weight. Data presented as the average percent weight loss within a given range. Post-WL = post weight loss.

All changes from baseline to post weight loss in lean mass and total and regional body fat were significant ($p < 0.05$). Total body fat mass decreased by approximately 5.7 kg or 16.6% and total body fat percentage decreased by 3.6% from baseline values. Android fat mass decreased by an average of 1.1 kg or 12.8%, while android fat percentage decreased by 4.7%. Similarly, a reduction of 1.3 kg or 8.8% was observed in gynoid fat mass. Gynoid fat percentage decreased 2.6%. Lastly, lean mass decreased by 1.7 kg or 2.7%. However, obese individuals increased lean mass percentage by approximately 3.5% from baseline. Please see tables 5a and 5b.

Table 5a. Changes in body fat and lean tissue distribution

	Baseline	2 – 4%	4 – 6%	6 – 8%	Post-WL
<u>Total mass</u>					
Lean (kg)	62.8 ± 4.1 ^{ab}	61.9 ± 3.9 ^{bc}	63.7 ± 4.1 ^a	61.5 ± 4.2 ^c	61.1 ± 4.2 ^c
Lean %	64.8 ± 1.4 ^a	66.4 ± 1.7 ^b	67.8 ± 1.8 ^c	68.4 ± 1.8 ^c	68.3 ± 1.7 ^c
Fat (kg)	34.4 ± 3.1 ^a	31.9 ± 3.3 ^b	30.7 ± 3.5 ^c	28.9 ± 3.4 ^d	28.7 ± 3.2 ^d
Fat %	35.2 ± 1.4 ^a	33.6 ± 1.7 ^b	32.2 ± 1.8 ^c	31.6 ± 1.8 ^c	31.6 ± 1.7 ^c
<u>Android mass</u>					
Fat (kg)	8.6 ± 0.8 ^a	8.1 ± 0.7 ^b	8.1 ± 0.7 ^b	7.5 ± 0.7 ^c	7.5 ± 0.7 ^c
Fat %	47.4 ± 1.8 ^a	45.7 ± 1.9 ^b	43.0 ± 2.1 ^c	43.0 ± 2.1 ^c	42.7 ± 2.0 ^c
<u>Gynoid mass</u>					
Fat (kg)	14.8 ± 1.0 ^a	14.2 ± 0.9 ^b	14.3 ± 0.9 ^b	13.6 ± 0.9 ^c	13.5 ± 0.9 ^c
Fat %	36.0 ± 1.7 ^a	34.9 ± 1.8 ^b	33.5 ± 1.7 ^c	33.3 ± 1.7 ^c	33.4 ± 1.7 ^c

All values presented as means ± standard error. Means with the same superscript are similar ($p > 0.05$).

Post-WL = post weight loss. Total mass = total body mass – bone mineral content. Android mass is defined by the following boundaries: lower = pelvic cut/superior angle of iliac crest; upper = 20% of the distance between pelvis and the neck cut/cervical vertebrae 5; lateral = medial border of the arm cuts.

Gynoid mass is defined by the following boundaries: upper/lower boundary = below pelvis by 1.5 x height of android ROI; lateral = outer leg cuts. Definition as described by GE Lunar Prodigy software.

Table 5b. Change values for body fat and lean tissue

	Baseline	2 – 4%	4 – 6%	6 – 8%	Post-WL
<u>Total mass</u>					
Lean (kg)	0 ^{a b}	-0.9 ^{b c}	0.9 ^a	-1.3 ^c	-1.7 ^c
Lean %	0 ^a	1.6 ^b	3.0 ^c	3.6 ^c	3.5 ^c
Fat (kg)	0 ^a	-2.5 ^b	-3.7 ^c	-5.5 ^d	-5.7 ^d
Fat %	0 ^a	-1.6 ^b	-3.0 ^c	-3.6 ^c	-3.6 ^c
<u>Android mass</u>					
Fat (kg)	0 ^a	-0.5 ^b	-0.5 ^b	-1.1 ^c	-1.1 ^c
Fat %	0 ^a	-1.7 ^b	-4.4 ^c	-4.4 ^c	-4.7 ^c
<u>Gynoid mass</u>					
Fat (kg)	0 ^a	-0.6 ^b	-0.5 ^b	-1.2 ^c	-1.3 ^c
Fat %	0 ^a	-1.1 ^b	-2.5 ^c	-2.7 ^c	-2.6 ^c

All changes calculated from initial baseline values (see table 5a). Baseline values indicated as “0”. Values with the same superscript are similar ($p > 0.05$). Post-WL = post weight loss. Total mass = total body mass – bone mineral content. Android mass is defined by the following boundaries: lower = pelvic cut/superior angle of iliac crest; upper = 20% of the distance between pelvis and the neck cut/cervical vertebrae 5; lateral = medial border of the arm cuts. Gynoid mass is defined by the following boundaries: upper/lower boundary = below pelvis by 1.5 x height of android ROI; lateral = outer leg cuts. Definition as described by GE Lunar Prodigy software.

Despite significant reductions in total body fat, BMI and waist girth after modest weight loss, these measures remained significantly higher when compared to the control participants at baseline ($p < 0.05$). However, differences in regional body fat that were significant between groups at baseline were no longer significant after modest weight loss in the obese group ($p > 0.05$).

Humoral Indices of Metabolic Homeostasis

NEFA’s, glucose, insulin, glucose to insulin ratio (GIR), the homeostasis model assessment (HOMA), and the quantitative insulin sensitivity check index (QUICKI) are included as markers of metabolic homeostasis. Glucose and NEFA concentrations were unaltered with modest 8 to 10% weight loss ($p > 0.05$). However, insulin, GIR, HOMA and QUICKI all were significantly improved by 8 to 10% weight loss ($p < 0.05$). Insulin

levels decreased by 5.9 $\mu\text{U}/\text{mL}$ or 24.3%. GIR increased by 1.31 or 32.0%. HOMA, a surrogate measure of insulin resistance, was reduced by 25.4% from 5.60 to 4.18. Finally, QUICKI, an indicator of insulin sensitivity, increased by 4.3%. The weight loss responses of these markers are provided in Tables 6a and 6b.

Table 6a. Weight loss responses in humoral indices of metabolic homeostasis

Variable	Baseline	2 – 4%	4 – 6%	6 – 8%	Post-WL
NEFA	0.70 \pm 0.09 ^a	0.73 \pm 0.15 ^a	0.68 \pm 0.12 ^a	0.76 \pm 0.15 ^a	0.67 \pm 0.06 ^a
Glucose	91 \pm 4 ^a	92 \pm 3 ^a	94 \pm 4 ^a	89 \pm 3 ^a	90 \pm 3 ^a
Insulin	24.3 \pm 3.2 ^a	22.6 \pm 2.9 ^{a,b}	21.3 \pm 2.9 ^b	18.2 \pm 2.6 ^c	18.4 \pm 2.3 ^c
GIR	4.09 \pm 0.35 ^a	4.43 \pm 0.34 ^a	4.82 \pm 0.44 ^{a,b}	5.33 \pm 0.55 ^b	5.40 \pm 0.55 ^b
HOMA	5.60 \pm 0.89 ^a	5.34 \pm 0.93 ^a	5.09 \pm 0.86 ^a	4.00 \pm 0.68 ^b	4.18 \pm 0.66 ^b
QUICKI	0.301 \pm 0.004 ^a	0.304 \pm 0.005 ^a	0.306 \pm 0.006 ^a	0.316 \pm 0.005 ^b	0.314 \pm 0.006 ^b

All values presented as means \pm standard error. Means with the same superscript are similar ($p > 0.05$).

Units: NEFA (mEq/L); glucose (mg/dL); insulin ($\mu\text{U}/\text{mL}$). Post-WL = post weight loss; GIR = glucose to insulin ratio; HOMA = homeostasis model score [fasting insulin $\mu\text{U}/\text{mL}$ x fasting glucose (mmol/L)]/22.5; QUICKI = quantitative insulin sensitivity check index [1 / (log (fasting insulin $\mu\text{U}/\text{mL}$) + log (fasting glucose mg/dL))].

Table 6b. Change values for humoral indices of metabolic homeostasis

Variable	Baseline	2 – 4%	4 – 6%	6 – 8%	Post-WL
NEFA (mEq/L)	0 ^a	0.03 ^a	-0.02 ^a	0.06 ^a	-0.03 ^a
Glucose (mg/dL)	0 ^a	1 ^a	3 ^a	-2 ^a	-1 ^a
Insulin (μU/mL)	0 ^a	-1.7 ^{a b}	-3.0 ^b	-6.1 ^c	-5.9 ^c
GIR	0 ^a	0.34 ^a	0.73 ^{a b}	1.24 ^b	1.31 ^b
HOMA	0 ^a	-0.26 ^a	-0.51 ^a	-1.6 ^b	-1.42 ^b
QUICKI	0 ^a	0.003 ^a	0.005 ^a	0.015 ^b	0.013 ^b

All changes calculated from initial baseline values (see table 6a). Baseline values indicated as “0”. Values with the same superscript are similar ($p > 0.05$). Post-WL = post weight loss; GIR = glucose to insulin ratio; HOMA = homeostasis model score [fasting insulin μU/mL x fasting glucose (mmol/L)]/22.5; QUICKI = quantitative insulin sensitivity check index [$1 / (\log (\text{fasting insulin } \mu\text{U/mL}) + \log (\text{fasting glucose mg/dL}))$].

Although changes in insulin, GIR, HOMA and QUICKI were significant ($p < 0.05$) in the obese group post weight loss, levels of these variables were still not similar to those observed in control participants at baseline ($p < 0.05$).

Significant correlations between alterations in body tissue stores and markers of metabolic homeostasis with weight loss are as follows ($p > 0.05$): total fat % and NEFA ($r = 0.78$); fat mass and NEFA ($r = 0.70$); gynoid fat and NEFA ($r = 0.83$); lean % and NEFA (-0.78); fat mass and glucose ($r = 0.73$); android mass and glucose ($r = 0.73$); lean mass and insulin ($r = 0.85$); android mass and HOMA ($r = 0.75$); lean mass and HOMA ($r = 0.89$); android mass and QUICKI ($r = -0.77$); gynoid mass and QUICKI ($r = -0.74$); lean mass and QUICKI ($r = -0.89$).

Cardiotrophin-1, Fibroblast Growth Factor-21 and Cytokine Responses

No significant change in cardiotrophin-1 concentrations occurred with 8 to 10% weight loss ($p > 0.05$). CT-1 concentrations significantly correlated with the following variables ($p < 0.05$): waist circumference ($r = .68$), VO_2max ($r = -0.82$), and glucose ($r = 0.86$). FGF-21 concentrations significantly decreased 57.3% ($p < 0.05$) after a weight

reduction of 8 to 10%. Please see figure 6. The changes in CT-1 and FGF-21 are described in Tables 7a and 7b.

Table 7a. CT-1 and FGF-21 responses to weight loss

Variable	Baseline	2 – 4%	4 – 6%	6 – 8%	Post-WL
CT-1	0.295 ± 0.029 ^a	0.294 ± 0.024 ^a	0.266 ± 0.029 ^a	0.271 ± 0.026 ^a	0.288 ± 0.039 ^a
FGF-21	433 ± 50 ^a	280 ± 59 ^{b c}	332 ± 67 ^{a b}	282 ± 76 ^{b c}	185 ± 34 ^c

All values presented as means ± standard error. Means with the same superscript are similar ($p > 0.05$).
Units: CT-1 (nmol/L); FGF-21 (pg/mL). Post-WL = post weight loss.

Table 7b. Change Values for CT-1 and FGF-21

Variable	Baseline	2 – 4%	4 – 6%	6 – 8%	Post-WL
CT-1 (nmol/L)	0 ^a	-0.001 ^a	-0.029 ^a	-0.024 ^a	-0.007 ^a
FGF-21 (pg/mL)	0 ^a	-153 ^{b c}	-101 ^{a b}	-151 ^{b c}	-248 ^c

All changes calculated from initial baseline values (see table 7a). Baseline values indicated as “0”. Values with the same superscript are similar ($p > 0.05$). Post-WL = post weight loss.

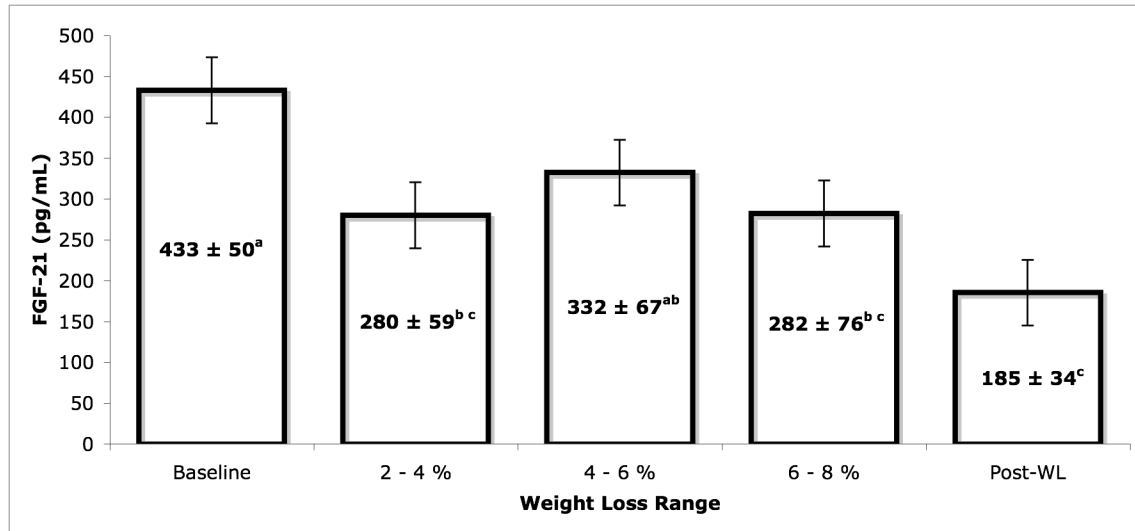


Figure 6. Changes in FGF-21 with weight loss. All values are means \pm standard error. Means with the same superscript are similar ($p > 0.05$). Post-WL = post weight loss.

Circulating concentrations of the cytokines adiponectin, TNF- α and IL-6 did not significantly change after an 8 to 10% weight reduction ($p > 0.05$). Adiponectin concentrations were significantly elevated ($p < 0.05$) post weight loss as compared to the 4 to 6% weight loss range; however, post weight loss concentrations were not significantly different from baseline values ($p > 0.05$). The responses of adiponectin as well as TNF- α and IL-6 are described in Tables 8a and 8b.

Table 8a. Cytokine responses to weight loss

Variable	Baseline	2 – 4%	4 – 6%	6 – 8%	Post-WL
Adiponectin	4047 \pm 571 ^{a,b}	4032 \pm 456 ^{a,b}	3833 \pm 483 ^b	3969 \pm 515 ^{a,b}	4546 \pm 599 ^a
TNF- α	1.35 \pm 0.12 ^a	1.34 \pm 0.08 ^a	1.15 \pm 0.11 ^a	1.07 \pm 0.14 ^a	1.11 \pm 0.11 ^a
IL-6	1.98 \pm 0.31 ^a	1.69 \pm 0.27 ^a	2.03 \pm 0.24 ^a	1.79 \pm 0.30 ^a	1.87 \pm 0.42 ^a

All values presented as means \pm standard error. Means with the same superscript are similar ($p > 0.05$).

Units: adiponectin (ng/mL); TNF- α (pg/mL); IL-6 (pg/mL). Post-WL = post weight loss.

Table 8b. Cytokine change values

Variable	Baseline	2 – 4%	4 – 6%	6 – 8%	Post-WL
Adiponectin (ng/mL)	0 ^{ab}	-15 ^{ab}	-214 ^b	-78 ^{ab}	499 ^a
TNF- α (pg/mL)	0 ^a	-0.01 ^a	-0.20 ^a	-0.28 ^a	-0.24 ^a
IL-6 (pg/mL)	0 ^a	-0.29 ^a	0.05 ^a	-0.19 ^a	-0.11 ^a

All changes calculated from initial baseline values (see table 8a). Baseline values indicated as “0”. Values with the same superscript are similar ($p > 0.05$). Post-WL = post weight loss.

Oxidative Stress Responses

Myeloperoxidase (MPO), an enzyme that catalyzes lipid peroxidation, thus contributing to oxidative stress, and total antioxidant capacity, a marker of defenses against reactive oxygen species and free radical damage, were measured as biomarkers of oxidative stress. Although MPO levels fluctuated with modest weight loss and were significantly ($p < 0.05$) reduced from 4 to 6% weight loss to post-weight loss, the change was not significant from baseline to post-weight loss ($p > 0.05$). These changes are illustrated in figure 7. Total antioxidant capacity was unaffected by modest weight reduction of 8 to 10% ($p > 0.05$). The changes in MPO and TAC are provided in Tables 9a and 9b.

Table 9a. Weight loss responses of MPO and TAC

Variable	Baseline	2 – 4%	4 – 6%	6 – 8%	Post-WL
MPO	257 \pm 20 ^{ab}	255 \pm 25 ^{ab}	282 \pm 27 ^a	247 \pm 23 ^b	225 \pm 23 ^b
TAC	334 \pm 11 ^a	339 \pm 8 ^a	336 \pm 8 ^a	340 \pm 11 ^a	336 \pm 11 ^a

All values presented as means \pm standard error. Means with the same superscript are similar ($p > 0.05$). Units: MPO (ng/mL); TAC (mmol trolox). Post-WL = post weight loss; MPO = myeloperoxidase; TAC = total antioxidant capacity; trolox equivalents are used to standardize antioxidants.

Table 9b. Change values for MPO and TAC

Variable	Baseline	2 – 4%	4 – 6%	6 – 8%	Post-WL
MPO (ng/mL)	0 ^{a b}	-2 ^{a b}	25 ^a	-10 ^b	-32 ^b
TAC (mmol trolox)	0 ^a	5 ^a	2 ^a	6 ^a	2 ^a

All changes calculated from initial baseline values (see table 9a). Baseline values indicated as “0”. Values with the same superscript are similar ($p > 0.05$). Post-WL = post weight loss; MPO = myeloperoxidase; TAC = total antioxidant capacity; trolox equivalents are used to standardize antioxidants

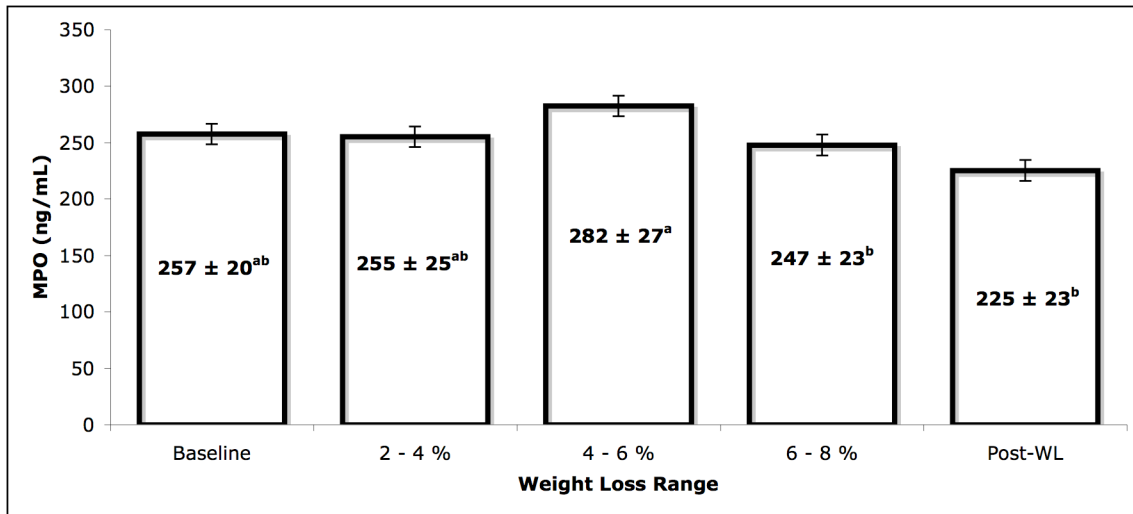


Figure 7. Changes in MPO with weight loss. All values presented as means \pm standard error. Means with the same superscript are similar ($p > 0.05$). Post-WL = post weight loss.

Physical Activity and Diet

Six of the nine obese individuals were assigned to the exercise based weight loss group. Exercise sessions were performed on a Trackmaster treadmill and were progressive and interval in nature. Each subject completed two supervised exercise sessions per week in the lab and was asked to aerobically exercise twice independently per week at approximately the same intensity based on heart rate ranges. Participants completed an average of 48 supervised exercise sessions or 84.4% of all possible sessions. Exercise sessions for each participant began with an initial caloric expenditure of 500 kcals. Caloric expenditure progressively increased to an 800 kcal maximum (as calculated from absolute oxygen consumption measurements) depending on the subject's

weight loss progression, ratings of perceived exertion and improvements in cardio-respiratory fitness. One participant completed the 8 – 10% weight loss goal at a final caloric expenditure of 800 kcals, one at 750 kcals, one at 725 kcals, two at 650 kcals and one at 625 kcals. Data from the first six and final six exercise sessions for each participant were averaged and used to determine progression and improvements that occurred due to the exercise intervention. The initial average duration for the first six exercise sessions was 46.4 minutes, while the average duration for the final six sessions was 52.8 minutes. Average intensity increased from 58.2% of heart rate reserve (HRR) over the first six sessions to 60.13% of HRR for the final six sessions. Moreover, intensity increased from, on average, 69.8% of VO_2 reserve (as calculated from initial VO_{2max}) to 93.9% at the completion of the weight loss phase.

Three of the nine obese individuals assessed were assigned to the diet based weight loss group. Exercisers were instructed not to change their diet or nutrient intake. On the other hand, dieters were asked not to alter their physical activity habits. The diet group was initially instructed to reduce daily caloric intake by 300 kcals. This caloric restriction increased to a maximum of 500 kcal/day through the intervention based on the individuals weight loss progress. For the purpose of statistical analysis the results from both the diet and exercise groups were combined due to no observable difference in the extent or pattern of weight loss between groups.

Coefficients of Variation for Biochemical Analyses

All samples for one individual were measured in single runs for each biochemical parameter. Therefore, inter-assay variation did not influence the temporal responses determined for each individual. The inter-assay coefficients of variation were as follows: FGF-21 – 2.89%; MPO – 2.41%; TAC – 3.41%; adiponectin – 29.93%; NEFA – 2.78%; glucose – 0.62%. The average intra-assay coefficients of variation were as follows: CT-1 – 0.5% to 5.2%; FGF-21 – 4.44%; MPO – 2.45%; TAC – 2.11%; adiponectin – 3.11%; NEFA – 3.07%; glucose – 1.80%; insulin – 2.93%.

Chapter V.

Discussion

Cytokines and adipokines influence physiological adaptations in obesity and during weight loss (37, 126, 143, 222). Both CT-1 and FGF-21 may be related to the metabolic dysfunction occurring with obesity as well as the metabolic and cardiovascular health improvements associated with weight loss (268, 365, 636, 645). Elevations in both CT-1 and FGF-21 in obese individuals have recently been described, yet changes in individuals undergoing weight loss are not currently known (365, 636). Therefore, our purpose was to determine changes in cardiotrophin-1 and fibroblast growth factor-21 in obese individuals undergoing modest weight loss of 8 to 10% of initial body weight. We hypothesized that 8 to 10% weight loss would significantly decrease circulating concentrations of CT-1 and FGF-21. Our findings indicate that FGF-21 concentrations are significantly reduced by 248 pg/mL or 57.3% after modest weight loss, while CT-1 levels were not significantly altered. The extent to which circulating CT-1 and FGF-21 concentrations reflect tissue metabolism or exert endocrine or paracrine effects similar to those observed in animal models and cell culture is yet to be determined. However, to our knowledge these are the first reported findings indicating the responses of circulating concentrations of CT-1 and FGF-21 to modest weight loss.

Changes in Fibroblast Growth Factor-21 with Weight Loss

There is currently little evidence in the literature regarding the influence of modest weight loss on circulating concentrations of FGF-21 in obese individuals. We hypothesized that weight loss of 8 to 10% of initial body weight would significantly decrease FGF-21 concentrations. In fact, we found that 8 to 10% weight loss results in a significant 57.3% reduction in FGF-21 levels. It has recently been determined that

adipose tissue is a source of FGF-21 expression possibly leading to elevated levels of this cytokine in obesity (636). Previous work demonstrates that there are differences in FGF-21 concentrations between obese and lean individuals (73, 308, 636). Elevated levels of FGF-21 are also reported in obese individuals and in persons with type 2 diabetes and MetS (73, 308, 636). Our findings suggest substantial individual variability in FGF-21 concentrations, such that FGF-21 levels were not different in obese individuals as compared to age-matched controls. We are the first to report, however, that modest weight loss of 8 to 10% of initial body weight results in significant reductions in FGF-21 concentrations.

2 to 4% weight loss caused an initial significant decrease in FGF-21 concentrations from baseline. Subsequently, FGF-21 levels increased through the 4 to 6% range followed by a continued reduction until the target weight loss of 8 to 10% of initial body weight was achieved. This response differed from our hypothesis that FGF-21 concentrations would initially decrease rapidly in response to our intervention followed by a more gradual decrease over time until the desired 8 to 10% weight loss was achieved. The rationale for this hypothesis was based on previous findings showing that modest weight loss caused initial rapid reductions in markers of oxidative stress that continued at a slower pace throughout the intervention (96, 318, 412, 478, 513, 571, 573).

The possible mechanisms explaining elevated FGF-21 concentrations observed with obesity in previous investigations are not completely understood (73, 308, 636). The elevations in FGF-21 levels may be an indirect sign of FGF-21 resistance, a protective mechanism or possibly indicate the presence of a truncated, inactive form of the protein in circulation (475). These elevated concentrations of FGF-21 would signify a protective mechanism if, in fact, FGF-21 elicits potent metabolic effects in humans as is observed in animal models (89, 268, 269). An increased expression of FGF-21 could be part of the body's natural defense against increased caloric intake that is associated with weight gain and obesity.

The significant reduction in FGF-21 that occurred with modest weight loss in our cohort could be due to the restoration of normal FGF-21 production and/or signaling, as occurs with insulin. Yet, if this was the case it seems that reductions in FGF-21 would more closely follow changes in insulin, GIR, HOMA and QUICKI. Changes in FGF-21

concentrations also appear to mirror the reductions in lean tissue mass, yet correlations between these two variables were not significant.

Metabolic efficiency may be defined as a physiological bias towards weight gain or a decrease in metabolic rate accompanying weight loss in order to defend the original body mass and account for increased caloric expenditure or decreased caloric intake. In other words, human bioenergetics are biased more to protect against weight loss than to prevent unwanted weight gain (341). It is known that lean tissue is more metabolically active than fat and that changes in metabolic efficiency occur with weight loss (341). FGF-21 also appears to be a potent metabolic regulator (89, 268, 269, 592). Thus, changes in both lean tissue and FGF-21 may serve as indicators of alterations in metabolic efficiency that occur with weight loss. As elevations or reductions in lean mass occur it is plausible that FGF-21 concentrations will increase or decrease concomitantly in order to accommodate the changes in metabolic demand. Findings from Dostalova and colleagues (111) provide evidence for this postulation. This group found significantly reduced FGF-21 concentrations in individuals with anorexia nervosa as opposed to normal weight controls (111). Anorexic individuals have little lean tissue mass and due to extremely low body weight and low caloric intake most likely demonstrate a higher degree of metabolic efficiency, which signifies a bias toward weight gain. Thus, the findings of Dostalova and colleagues (111) provide evidence supporting the possibility that FGF-21 concentrations may indeed track changes in lean tissue mass and metabolic demand.

As noted previously, significant differences in circulating FGF-21 concentrations between obese and lean individuals have been reported (73, 308, 636). However, our data do not corroborate these findings. If adhering to the notion that changes in FGF-21 track alterations in lean tissue mass and metabolic demand, then the lack of a noteworthy difference between FGF-21 levels in the obese and control individuals in our cohort makes sense due to the fact that there also was not a significant difference in lean tissue mass between the two groups. This provides possible evidence that alterations in lean tissue mass may influence changes in FGF-21 with weight loss.

Changes in Cardiostrophin-1 with Weight Loss

Recent data show that CT-1 is expressed in adipose tissue and is up-regulated in states of obesity and in individuals with MetS (334, 365). Moreover, CT-1 inhibits adipocyte insulin signaling and insulin-stimulated glucose uptake in cell culture (645). CT-1 also has well documented effects on the myocardium that include left ventricular hypertrophy and prevention of cardiac myocyte death in response to myocardial ischemia (299). Elevated CT-1 levels are found along with failing left ventricular myocardium, left ventricular hypertrophy, treated and untreated hypertension, and vascular dysfunction (319, 320, 334, 421, 453, 644). Therefore, it was thought that cardiostrophin-1, through increased adipose tissue expression in obese individuals, may take part in the link between obesity, insulin resistance and cardiovascular pathologies such as hypertension, left ventricular hypertrophy and congestive heart failure. Based on previous findings and this rationale, we hypothesized that CT-1 concentrations in our obese cohort would be elevated as compared to control individuals and that 8 to 10% weight loss would significantly reduce circulating levels of this bioactive peptide.

However, it does not appear that CT-1 concentrations are significantly different between obese and control individuals in our cohort. Furthermore, CT-1 concentrations did not change with weight loss of 8 to 10% of initial body weight. Natal and colleagues (365) reported that adipose tissue expresses CT-1 in 3T3-L1 cell culture, murine and human adipose tissue. However, the adipose expression as compared to other tissues was only investigated in the murine model. Therefore, human adipose tissue may in fact be a source of cardiostrophin-1, yet this bioactive protein may be expressed to a greater degree in other tissues such as the heart. Studies investigating the expression of CT-1 in various human tissues need to be performed before it can be determined if adipose tissue is a major source of this cytokine in humans.

Zvonic et al. (645) reported that 0.2 nM CT-1 treatment significantly reduced insulin-stimulated glucose uptake in 3T3-L1 adipocytes and hypothesized that CT-1 may be a mediator of impaired insulin sensitivity. The 0.2 nM treatment levels were similar to the fasting serum concentrations of CT-1 found in our cohort. However, our data do not indicate a relationship between CT-1 and insulin resistance. CT-1 concentrations did not change with weight loss or improvements in insulin sensitivity and did not significantly

correlate with any clinical surrogate markers of insulin sensitivity. This discrepancy may be due to the fact that Zvonic and colleagues (645) reported findings from 3T3-L1 adipocytes in vitro. Thus, future studies in vivo are necessary to determine if CT-1 does indeed contribute to obesity related insulin resistance in humans.

Moreover, Natal et al. (365) showed that cardiostrophin-1 expression in 3T3-L1 adipocytes was dose-dependently related to glucose and that CT-1 positively correlated with glucose concentrations in human serum. In fact, we too found a significant correlation ($r = 0.86$, $P = 0.003$) between cardiostrophin-1 and glucose levels. However, none of the individuals in our cohort had diabetes or severe impaired fasting glucose. Thus, it may be that CT-1 concentrations are elevated to a greater extent in obese individuals with impaired fasting glucose or type II diabetes mellitus and that modest weight loss has a greater affect on CT-1 concentrations in these individuals. In other words, it is possible that since we observed no weight loss induced changes in fasting blood glucose concentrations, then CT-1 concentrations were unaltered as well.

Weight Loss and Insulin Sensitivity

The modest weight loss achieved through this intervention caused significant reductions in overall body fat, particularly body fat in the android and gynoid regions. Lean mass was significantly reduced, although when expressed as a percentage of total body mass, lean mass increased from baseline to target weight loss. This indicates that a majority of the observed weight loss was through reductions in body fat. Body composition changes, apart from those observed in lean mass, occurred sequentially (see Table 5). Obese individuals lost an average of 8.9 kg with contributions of 5.7 kg, 1.7 kg, 1.1 kg and 1.3 kg coming from total fat mass, lean mass, android fat and gynoid fat, respectively. From a percentage standpoint losses in total fat mass, android and gynoid mass were much more substantial as lean mass percentage actually increased. Yet, it still remains that losses in both fat mass and lean tissue mass contributed to weight loss. These findings are similar to those reported previously for obese individuals (74, 172, 468, 522). It appears that modest weight loss consistently results in significant reductions in total body fat, android fat, gynoid fat and lean tissue. Exercise is often credited with preserving lean tissue if practiced while undergoing weight loss; however, this is an

equivocal finding (98, 272). In our cohort lean mass decreased in spite of the regularly practiced exercise regimen by most of our participants.

The weight loss achieved through this intervention induced significant reductions in fasting insulin concentrations and clinical surrogate markers of insulin sensitivity. With weight loss, both GIR and QUICKI increased, indicating enhanced insulin sensitivity and HOMA decreased, indicating reduced insulin resistance. These findings are consistent with previous results in obese individuals, whereby HOMA decreased and QUICKI increased with modest weight loss (113, 452, 491). In the present cohort, significant reductions in insulin concentrations occur with only 4 to 6%. This indicates that metabolic improvements begin with only slight weight loss. However, significant changes in the GIR, HOMA, and QUICKI index are not evident until 6 to 8% weight loss is achieved.

Enhanced insulin sensitivity after weight loss is partially related to the loss of total body fat and highly correlated with the loss of android fat (172). Similarly, changes here were accompanied by significant decreases in total and android body fat. No significant correlations were found between total body fat reductions, insulin and surrogate markers of insulin sensitivity. However, significant relationships were observed between regional body fat stores, HOMA and QUICKI, as well as between lean mass, HOMA and QUICKI. Thus, in our cohort it appears that changes in insulin sensitivity and insulin action reflect those seen in regional body fat and lean mass. The strongest relationships occur between alterations in markers of insulin sensitivity and lean body mass, which is not reported prevalently in the literature. The strong correlation between surrogate markers of insulin sensitivity and lean mass reduction may seem counter-intuitive, as muscle is more metabolically active than fat tissue. However, this may be due to a concomitant decrease in intramuscular lipid content, known to promote insulin resistance and decrease with weight loss (131, 162). Yet, we currently have no data to support this postulation.

Modest weight loss and changes in body composition frequently result in decreased fasting glucose concentrations (4, 144, 202, 599, 602). Yet, fasting glucose was unaffected by our intervention. This may be due to the fact that the mean fasting glucose concentration of our cohort at baseline was 95 mg/dL, which is below the > 100

mg/dL indicator for impaired fasting glucose and within the normal fasting glucose range. The significant changes frequently reported in the literature usually occur with weight loss in obese individuals with impaired fasting glucose or type 2 diabetes at baseline (4, 202, 261, 262, 339, 583, 599).

Our findings are fairly consistent with previous reports that weight loss does not need to be drastic, as modest weight reductions of 5 to 10% can significantly improve insulin action, glucose metabolism and hyperinsulinemia in obese individuals (170, 276, 390, 413, 599, 602). The present data indicate that our intervention induced weight loss adequate to elicit beneficial changes in metabolic regulation and manifested as improvements in insulin concentrations and surrogate markers of insulin sensitivity. These changes are reflective of alterations in body composition and body tissue stores, notably regional body fat and lean mass. Modifications in body tissue stores and markers of metabolic homeostasis occurred rapidly within the weight loss process, usually within the 4 to 8% weight loss range. This further solidifies the concept that only modest weight loss below 10% of initial body weight is necessary to induce positive anthropometric and metabolic outcomes.

Outside Influences on These Findings

Factors that may have influenced the results of this investigation include diet (nutrient composition and caloric intake), outside physical activity, stress levels and sleep habits. Participants in the exercise group were advised throughout the study not to make significant changes in their diet. Each individual recorded all food and drink consumed for three days each month during the intervention. Food record analysis did not show any major changes in dietary nutrient composition or caloric intake. Furthermore, changes in total antioxidant capacity would reflect a change in diet consisting of increased intake of fruits and vegetables, which contain high levels of antioxidants. This type of change in nutrient consumption is common in dieting individuals. The fact that TAC remained stable throughout the intervention indicates that changes in nutrient intake most likely did not occur. During the 6 to 10 month intervention period individuals in the diet group were asked to avoid regular physical activity as much as possible. These participants had relatively sedentary jobs and were capable of maintaining their physical activity to typical

activities of daily living. All refrained from any formal exercise as indicated by monthly 3 day physical activity logs. It appears that no major changes in dietary composition occurred in the exercise group and that no significant elevations in physical activity level occurred in the diet group or were an appreciable influence on the magnitude or direction of blood variable changes observed with weight loss. Stress levels and sleep habits were not directly controlled or quantified during the intervention.

Overall Findings

The results of this investigation suggest that modest weight loss of 8 to 10% of initial body weight is effective in reducing circulating concentrations of FGF-21. However, weight loss did not alter cardiostrophin-1 concentrations. FGF-21 levels were significantly reduced by 57.3% from baseline to post weight loss. 2 to 4% weight loss caused an initial significant reduction in FGF-21 concentrations followed by an increase through the 4 to 6% range. Subsequently, FGF-21 levels were significantly reduced in the target weight loss range of 8 – 10%.

Furthermore, weight loss resulted in a 2.7% decrease in lean tissue mass. The fact that both lean tissue mass and FGF-21 concentrations were reduced after 8 – 10% weight loss is interesting since lean tissue is more metabolically active than fat and FGF-21 is described as a potent metabolic regulator (89, 268, 269, 355, 592). In other words, fluctuations in FGF-21 and lean tissue may reflect alterations metabolic demand occurring with weight loss. Melby and Hickey (341) have previously described the concept of metabolic efficiency. Losses in lean tissue mass and reductions in FGF-21 may contribute to increased metabolic efficiency with weight loss. The elevated FGF-21 concentrations previously reported in obese individuals may be associated with elevated lean tissue mass, not excess adipose tissue, FGF-21 resistance, a protective mechanism, or production of truncated, inactive forms of the protein (73, 308, 636). Thus, the observed reductions in FGF-21 concentrations with 8 to 10% weight loss may be a result of lean mass loss and enhanced metabolic efficiency. However, this is a postulation and is not the result of mechanistic data.

Other possible explanations exist as to why FGF-21 decreases with weight loss. If there is in fact some level of FGF-21 resistance in obese individuals, much like is

known to occur with insulin, then the reductions in FGF-21 observed with modest weight loss may be reflective of the restoration of normal FGF-21 production and signaling. Furthermore, reductions in FGF-21 occurred along with significant body fat loss. Therefore, decreased adipose tissue mass and restoration of adipose tissue health may possibly cause fat tissue to become less resistant to the effects of FGF-21.

Clinical Significance and Conclusions

Finally, to our knowledge we are the first to report the effects of modest weight loss on circulating concentrations of cardiostrophin-1 and fibroblast growth factor-21. CT-1 levels most likely are quite clinically important based on this bioactive peptide's well-documented effects on the heart (299, 319, 321, 334, 421, 453, 644). However, our data suggest that the effect of modest weight loss on concentrations of CT-1 holds little clinical significance in obese individuals without impaired fasting glucose.

If the hypothesis is true that FGF-21 concentrations may track changes in lean tissue and metabolic demand, the clinical relevance of this cytokine may be substantial. The increased concentrations of FGF-21 in obese individuals reported previously may be due to elevated lean tissue mass as opposed to increased adipose tissue expression and subsequent FGF-21 resistance (73, 308, 636). Thus, FGF-21 could be a potential therapeutic and pharmaceutical target that along with proper diet and exercise may be capable of eliciting potent metabolic benefits in obese individuals. However, a wealth of further data is needed before this postulation can be solidified.

From a clinical standpoint, it is well documented that weight loss results in a number of metabolic benefits including reductions in body fat and enhanced insulin sensitivity (74, 113, 172, 452, 467, 491, 522). The conservative weight loss regimen employed in this study lead to significant reductions in total, android and gynoid body fat stores of 5.7 kg, 1.1 kg and 1.3 kg, respectively. Significant beneficial changes in fasting insulin concentrations and clinical surrogate markers of insulin sensitivity, such as GIR, HOMA and QUICKI were also observed. Some of these improvements in the clinical markers of insulin sensitivity began with as little as 4 to 6% weight loss, indicating that only slight weight loss is necessary to induce metabolic benefits. Thus, our findings corroborate a wealth of previous information citing that modest weight loss through

lifestyle modifications is effective in causing changes in total and regional body fat as well as clinical markers of metabolic health (74, 113, 170, 172, 276, 390, 413, 452, 467, 491, 522, 599, 602). These changes reflect a reduction in the risk for MetS, type II diabetes mellitus and cardiovascular disease (100, 180, 182, 293, 296, 597, 631). In conclusion, modest weight loss is successful in causing significant and beneficial changes in total and regional body fat as well as in clinical markers of metabolic health.

According to data from this investigation, alterations in oxidative stress, markers of systemic inflammation and cardiotrophin-1 do not occur at the same time as improvements in glucose metabolism and insulin sensitivity happen with weight loss. However, reductions in FGF-21 do occur with the same 8 to 10% modest weight loss, signifying that FGF-21 may be a potential clinical indicator of improvements in metabolic regulation that occur with weight loss.

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