

Leptin Resistance and Left Ventricular Hypertrophy in Obese Children and Adolescents

By

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## Abstract

This study explores the relationship between leptin resistance (LR), as identified by elevated leptin levels, and cytokines, in obese children and adolescents. It is based on a conceptual framework that relates left ventricular hypertrophy (LVH) in obese children and adolescents to LR. LR is, perhaps, directly related to interleukin-1 receptor antagonist (IL-1RA), a potent anti-inflammatory cytokine. Regression analysis was performed with left ventricular mass (LVM) functioning as the dependent variable while age, sex, body mass index (BMI), mean arterial pressure (MAP), and serum levels of leptin, insulin, and IL-1RA as independent variables ( $R^2 = .41$ ,  $F(7, 12) = 1.204$ ,  $p = .37$ ). Because statistical power was not achieved, Cohen's guidelines for  $r$  were followed. Several correlations demonstrated moderate to large effects. The results showed that LVM correlated with BMI, suggesting a moderate effect ( $r = .46$ ). Other moderate correlations with BMI were detected with age ( $r = .77$ ), MAP ( $r = .50$ ), leptin ( $r = .56$ ), and insulin ( $r = .61$ ). The MAP for the sample population yielded correlations of moderate effect with leptin ( $r = .50$ ), insulin ( $r = .57$ ), and correlated strongly with IL-1RA ( $r = .75$ ). Insulin levels were also correlated with leptin ( $r = .64$ ) and IL-1RA ( $r = .52$ ), both moderate effects sizes. The findings supported previous studies in which elevated BMI was associated with LVH, elevated MAP, elevated leptin, and increased insulin levels. The moderately correlated effect between LVM and MAP contradicts results from some studies reporting that blood pressure in adults was not associated with LVH. Because no relationship was detected between leptin and IL-1RA, a mediator relationship could not be tested. No statistically significant interaction between LR and IL-1RA was detected. However, the findings support previous research suggesting that LVH in obese children is associated with an increase in MAP but not leptin. This suggests that changes in MAP may be related to increased IL-1RA .

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## Chapter 1

### Introduction

The United States Centers for Disease Control (CDC) defines obesity as an anthropometric measurement in children and adolescents as having “a gender- and age-specific body mass index (BMI) at or above the 95<sup>th</sup> percentile” of the general population (National Institute of Medicine Preventing Childhood Obesity, 2005). Overweight is defined as having a gender- and age-specific BMI ranking equal to or greater than the 80<sup>th</sup> percentile of the population. Obese children have been found to have increased blood pressure (BP), left ventricular hypertrophy (LVH), and heart chamber enlargement (Daniels, Witt, Glascock, Khoury, & Kimball, 2002; Gutin, Treiber, Owens, & Mensah, 1998; Hanevold, Waller, Daniels, Portman, & Sorof, 2004). Obesity in children and adolescents has also been associated with decreased high-density lipoprotein (HDL) levels, insulin resistance, and endothelial dysfunction (Jancin, 2003). Epidemiological studies have shown that some obese children are now developing type 2 diabetes mellitus (DM-2) and hyperlipidemia (Jancin, 2003). These conditions are suspected to be precursors to or associated with similar physiologic mechanisms and disease processes found in adults with metabolic syndrome.

One estimate suggests that obesity accounts for nearly 9% of United States healthcare spending (Finkelstein & Feibelkorn, 2003)(National Institute of Medicine Preventing Childhood Obesity, 2005). A decade ago, statistics suggested that obesity-related disease accounted for up to 6% of total healthcare expenditures (Seidell, 1998). Hering and colleagues reported on a study of 363 obese children matched with 382 children of normal weight found that the obese children had more hospitalizations, for a longer duration and were given more medications (Hering, Pritsker, Gonchar, & Pillar, 2009). The epidemic of obesity has rapidly gained recognition as an

international public health crisis due to its associated co-morbidities which include DM-2, coronary artery disease, peripheral vascular disease, stroke, hypertension, renal failure and osteoarthritis.

Left ventricular hypertrophy (LVH) is just one consequence of obesity. It refers to the condition when the muscle of the left ventricle of the heart is thicker than normal, resulting in increased myocardial oxygen demand and decreased cardiac output. Long-known to be associated with obesity in adults, LVH has recently been found to exist in obese children and adolescents as well (Hanevold et al., 2004). Several studies have suggested that LVH in adults is related to an inability of obese humans to properly use the hormone leptin (Pladevall et al., 2003). In obese persons, the inability to properly use leptin results in elevated levels of leptin circulating within the body. This phenomenon is known as leptin resistance (LR).

Scientists assess LVH by measuring the left ventricular mass (LVM) of the patient. To date, no studies have been conducted to explore whether LVM levels are associated with serum leptin levels in obese children. The current body of scientific literature lacks in several regards concerning child and adolescent obesity. This study attempts to address two gaps within the literature: to confirm whether or not LVH in obese children and adolescents is associated with LR and to explain why LR occurs in obese children.

To address the question of why LR occurs in obese children, this study was designed to explore the relationships between LR, cytokines, and LVH in obese children and adolescents. This study design draws upon the theory that LVH in obese children and adolescents occurs as a result of LR. A premise of this research is that LR may be directly related to elevated levels of interleukin-1 receptor antagonist (IL-1RA), a potent anti-inflammatory cytokine. Because LVH in obese individuals was historically considered related to elevated BP, the first part of this

research was performed where LVM was found to be related to BP, and IL-1RA accounted for a large proportion of the variance in BP. Recent studies have shown that IL-1RA is associated with mean arterial pressure (MAP) and LVH (Litwin et al., 2006; Norton et al., 2009). The specific aims of the current study included the following:

### *Specific Aims*

1. Detect whether or not leptin explains a statistically significant proportion of the variance of LVM in obese children and adolescents;
2. Detect whether or not IL-1RA is related to LVM and if leptin mediates this relationship in obese children and adolescents;
3. Detect whether or not MAP accounts for a statistically significant proportion of the variance in LVM in children and adolescents; and to
4. Determine if IL-1RA explains a statistically significant proportion of the variance in MAP in children and adolescents.

### *Definition of Terms*

Left Ventricular Hypertrophy (LVH) – Cardiac muscle cells do not increase in number after fetal development; therefore when additional stress is placed on cardiac muscle the muscle cells increase in size larger than normal. LVH is a compensatory mechanism. Just as skeletal muscle increases in size to compensate for increased stress, cardiac muscle increases in size in order to compensate for increased stress. Increased myocardial cell size results in increased cardiac wall thickness, which in turn causes decreased cardiac output, increased myocardial oxygen demand, and ultimately heart failure. LVH is calculated by measuring LVM, which is measured through a 2-dimensional echocardiogram of the left ventricular internal dimension,

interventricular septal thickness, and posterior wall thickness (Hanevold, et al., 2004). The equation for LVM (g) = 0.81 [1.04 (interventricular septal thickness + posterior wall thickness + LV end diastolic internal dimension)<sup>3</sup> - (LV end diastolic internal dimension)<sup>3</sup>] + 0.06 (Hanevold et al., 2004).

Leptin – In the mid-1990's a bioactive protein called leptin was discovered. Zhang et al., 1994. Leptin is produced by adipose cells, and acts at the hypothalamus to decrease food intake by stimulating the release of pro-opiomelanocortin and increase thermogenesis (Tartaglia, 1997). In starvation, leptin levels are very low. When leptin is secreted, appetite is decreased and metabolism increased. Leptin is therefore the body's mechanism to prevent obesity.

Leptin Resistance – Describes the phenomenon in humans whereby, despite elevated circulating levels of serum leptin, the individual does not have the expected physiologic responses of anorexia and increased basal metabolic rate.

Interleukin-1 receptor antagonist (IL-1RA) – Refers to an anti-inflammatory cytokine produced abundantly by white adipose tissue (Somm et al., 2005). IL-1RA binds to the pro-inflammatory cytokine IL-1 $\beta$ , blocking its activity (H. Yang et al., 2005).

Mean arterial pressure (MAP) – MAP is used as a standardized method of assessing an average of systolic and diastolic BPs. It is the average arterial BP during a cardiac cycle. Systolic BP is the internal arterial pressure that occurs when the heart is contracting (systole). Diastolic BP is the internal arterial pressure that occurs when the heart is at rest (diastole). Mean arterial pressure is approximated by (2 x diastolic pressure) + systolic pressure divided by 3.

## Chapter 2

### Background and Significance

#### *Prevalence*

The prevalence of overweight and obese children and adolescents is currently increasing at an alarming rate within the general population of the United States. Statistics derived from the National Health and Nutrition Examination Survey (NHANES) IV (2010) suggest nearly 17% of all of children and adolescents aged 6 to 19 years are overweight (CDC, 2010). This represents an increase of over 50% from a similar assessment done in 1994 and a 228% increase from an assessment survey completed in 1980 (CDC, 2010).

#### *Problem*

While the rate of obesity rapidly increases in the general population, research studies have only scratched the surface as to the physiology of obesity-related disease. The physiology associated with obesity-related disease among children and adolescents also lacks complete description. Left ventricular hypertrophy—the thickening of the heart muscle—is one documented phenomenon found in obese children and adolescents that has previously been detected in obese adults (Kono et al., 1994; Yoshinaga et al., 1995). This location-specific thickening of the myocardial tissue shares a relationship with LR in obese adults (Pladevall et al., 2003). Leptin resistance is considered a condition in which the body becomes opposed to leptin at the cellular level. No method is currently known for directly measuring LR. The concept of LR is theoretical, based on the physiologic principal that fat cells produce leptin to signal the hypothalamus to increase metabolism and decrease appetite. In obese humans, leptin levels are very high, yet there is no decrease in appetite or increase in metabolism (Banks, 2008).

Therefore, the anticipated physiologic effects of leptin are not present. This is why elevated leptin levels in an obese adult are considered to be due to LR (Banks, 2008).

LR is an assumption that is made when an obese person has elevated levels of leptin without the physiologic effects. Additionally, because leptin has been shown to be independently responsible for LVH in obese animals (Barouch, Berkowitz, Harrison, O'Donnell, & Hare, 2003) and adult humans (Kartal, Inal, Baysan, & Saglam, 2008), measuring LVH when leptin levels are elevated is considered an indirect measurement of LR. To examine LR in obese children, the relationships between leptin and LVH in obese children and adolescents are examined closely. No study has previously documented the relationships between leptin or LR and LVH in obese children and adolescents.

The cytokines interleukin-1 (IL-1), IL-6, IL-10, and IL1-RA may be associated with obesity-related diseases such as metabolic syndrome (K. C. Huang et al., 2004; Mendall et al., 1997; Okamoto et al., 2000). No studies appear in the body of scientific literature having identified how chronic inflammation affects heart size in obese children and adolescents. A principal component of the current study was detecting whether or not LR is linked to cytokine function in adult human and animal models (Munzberg, Bjornholm, Bates, & Myers, 2005; H. Yang et al., 2005). No study could be found within the body of literature that documents LR in children attenuated by cytokines. This study was not conducted to document LVH's cause rather it was to look at leptin resistance. LV-Mass was selected as the way to measure leptin resistance. A hypothesis of this research is that IL-1RA, a potent anti-inflammatory cytokine, plays an integral role in leptin resistance.

## *Leptin*

Leptin was first described in the mid 1990's as a hormone acting at the hypothalamus to regulate food intake and thermogenesis (Sato et al., 1997). Leptin is produced by adipose cells. Therefore, more adipose tissue should mean a higher level of circulating leptin in the body (Banks, 2008). In starvation the number of adipose cells decreases, leptin production decreases, and subsequently appetite is increased while metabolism is decreased. Leptin is known to be associated with many peripheral mechanisms such as immunity, bone mineralization, cognition and neurogenesis, and breathing (Banks, 2008). Leptin receptor genes contain genetic codes for several versions of the receptor molecule. The leptin receptor is particularly abundant in the hypothalamus (Tartaglia, 1997).

Multiple forms of the leptin receptor have been identified; the most active form is known as the long form LRb (Tartaglia, 1997). The leptin receptor has been found in the IL-6 family of class 1 cytokine receptors (Tartaglia, 1997). The LRb signals via a non-covalently associated tyrosine kinase molecule of the Janus Kinase (Jak2) family of receptors (Munzberg, et al., 2005). It is believed that the neuroendocrine function of leptin is based in the arcuate, dorsomedial, and ventromedial nuclei (Munzberg et al., 2005). In the arcuate nucleus LRb is found within 2 populations of neurons. The first inhibits neuropeptide Y and agouti related peptide (AgRP). The second synthesizes pro-opiomelanocortin (POMC), which is processed to produce  $\alpha$ -melanocyte-stimulating-hormone (Munzberg et al., 2005). This hormone signals anorexia. Neuropeptide Y acts within the brain to stimulate feeding (orexigenic) and decrease energy expenditure (Griffin & Ojeda, 2004). Agouti related protein is an antagonist to  $\alpha$ -melanocyte-stimulating-hormone ( $\alpha$ -MSH) (Munzberg et al., 2005). These interactions act as a feedback mechanism to alter appetite.

Three distinct pathways of leptin signaling have been identified in the neuroendocrine system, all of which appear to be dependent on the Jak2 pathway, including the phosphorylation of 2 tyrosine residues: Tyr985 and Tyr1138 (Munzberg et al., 2005). Tyr1138 stimulates the transcription of POMC. The Jak2, Tyr985, and Tyr1138 pathways are all attenuated by suppressor of cytokine signaling (SOCS3) pathway. POMC activates SOCS3 (Munzberg et al., 2005). Therefore, as anorexia is stimulated by the production of POMC, a feedback loop is activated where the LRb receptor is blocked and ultimately appetite is stimulated.

### *Leptin Resistance*

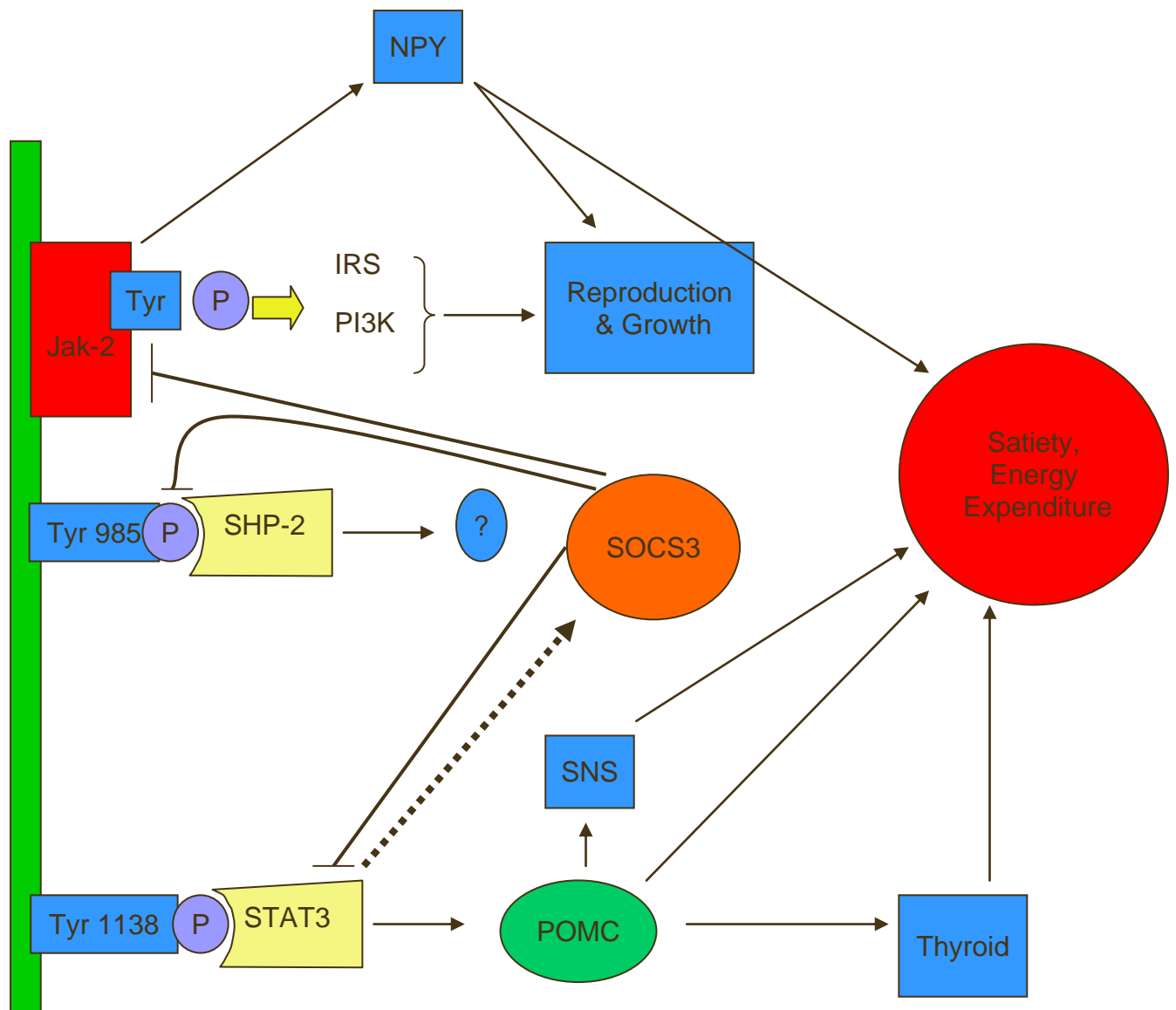
The discovery of leptin combined with related human studies provide an adequate description of the phenomenon of LR. This term encompasses a complex interaction of pathophysiological mechanisms. Central LR is a process whereby leptin reception is inhibited at the hypothalamic level. This process affects the physiologic mechanisms described earlier with metabolism. Peripheral LR has can injure peripheral tissues and systems including, liver, pancreas, platelets, vasculature, and myocardium (Martin, Qasim, & Reilly, 2008).

Leptin resistance is dependent on a complicated interplay of pathways. Because leptin functions in many body tissues, a single explanation for LR may not be appropriate. Banks (2008) states that LR may be part of three mechanisms including the blood-brain barrier (BBB), receptor/post-receptor activation, and a downstream neuronal circuit (pp. 1607). Martin and colleagues described five possible mechanisms of LR including circulating regulators, self regulation, genetic mutation, limited tissue access, and cellular regulators (Martin et al., 2008).

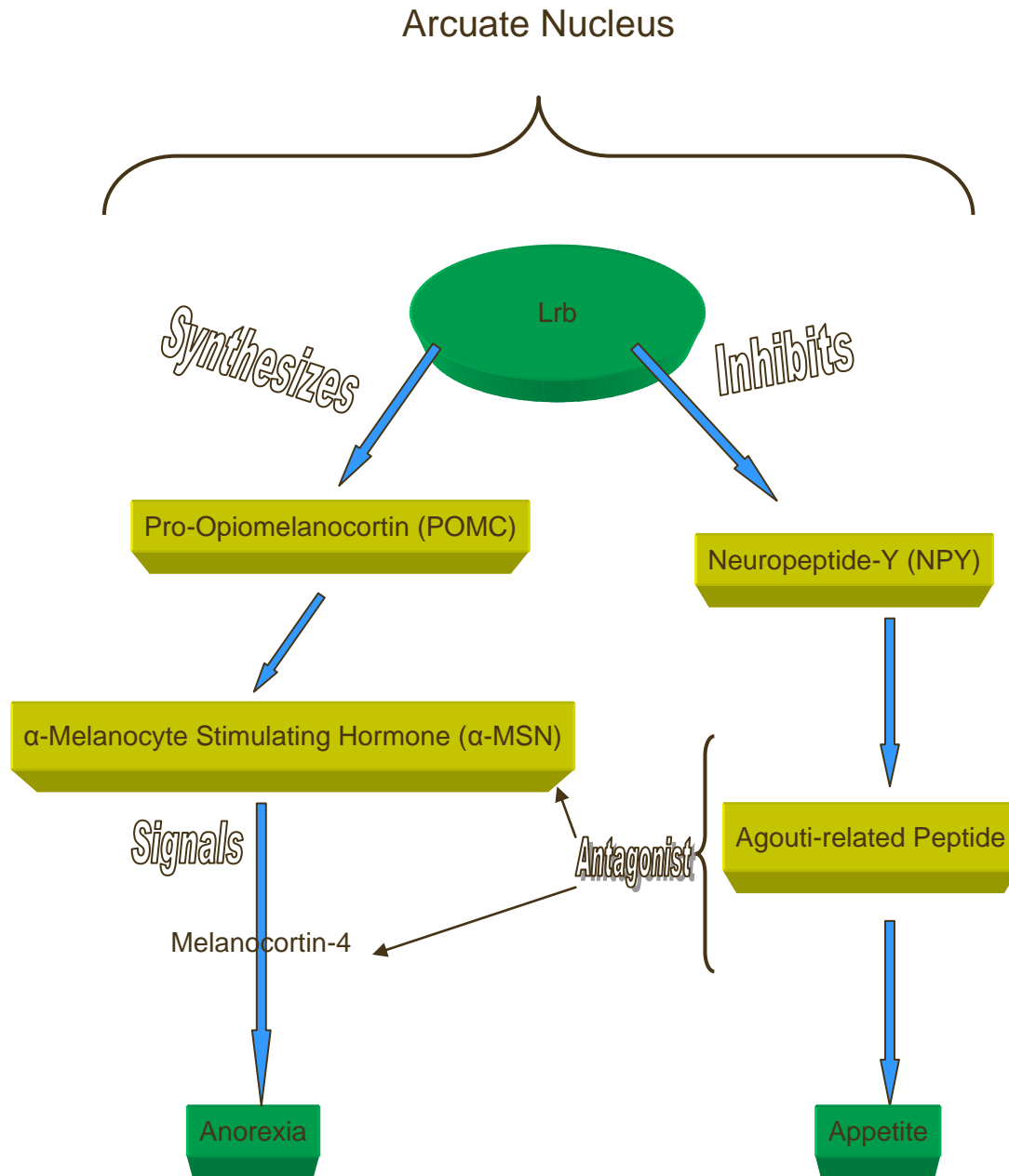
A genetic mutation for LR has been found and demonstrated in animal models (the Ob/Ob mouse model). Similar dysfunction has also been seen in humans, but is extremely rare (Considine et al., 1996). It is extremely unlikely that a genetic mutation would be acquired by



such a large and growing percentage of the population over a short period of time according to Mendelian principles. Martin and coauthors proposed that that polygenetic-inheritance patterns may still play a role in LR, but have yet to be adequately explained (Martin et al., 2008).



**Figure 1:** Model 1 for activation of the leptin receptor and pathway of anorexia versus appetite stimulation.



**Figure 2:** Model 2 of activation of the leptin receptor and pathway of anorexia versus appetite stimulation.

Resistance at the BBB has been described (Hileman et al., 2002). The short form of the Ob-R receptor appears to be associated with leptin reception at the blood brain barrier in rodents (Hileman et al., 2002). A principal objective of their study was to determine the relative expression of the short Ob-R iso forms at the blood-brain barrier. Another objective was to

determine whether Ob-R iso forms were required for normal brain uptake of leptin. Reported results demonstrated uptake of leptin by the brain was decreased in both polygenic and a diet-induced obese rodents (Hileman et al., 2002). This information suggests LR at the BBB.

Research evidence suggests that LR may be associated with a self-regulatory mechanism (Martin et al., 2008). Zhang and Scarpace (2006) discussed three mechanisms of central LR associated with self-regulation: an inability of peripheral leptin to reach the hypothalamus, decreased hypothalamic leptin receptor number and an impaired leptin signal transduction (Zhang & Scarpace, 2006). In a series of studies, the pair of investigators described how these scenarios for LR were demonstrated (Zhang & Scarpace, 2006). The first study described young lean rats compared to genetically-modified rats designed to acquire adult onset obesity, similar to what occurs in humans. The aged obese rats and lean controls were both given a central and peripheral leptin infusion. The results demonstrated a “markedly blunted” response in the aged-obese rats to both central and peripheral leptin administration. This response was measured by evaluating levels of STAT3 phosphorylation and P-STAT3 transcription factor binding to test hypothalamic signaling (pp. 250).

The authors also reported a study in which diet-induced down regulation of leptin receptors was induced by a high-fat diet. The study rats which were fed a high-fat diet were compared to rats which were fed a balanced diet. These two groups were then given a dose of leptin. This generated a greater than 6-fold increase in leptin STAT3 phosphorylation as compared to only a 2-fold increase in the obese rats fed a high fat diet. The obese rats were then placed on a calorie-restricted diet and subsequently given the same dose of leptin. The animals demonstrated a similar leptin response to the non-obese rats with a balanced diet. These results suggest that leptin receptors are down regulated in high-fat diet-induced obesity. The researchers

stated that this may be an instance of leptin acting as a “self-regulator” (Zhang & Scarpace, 2006).

The group also conducted a third study to determine whether regulated leptin transgene expression achieved the same degree of leptin signaling as seen in chronic transgene expression. The researchers designed a rat model that allowed regulation of hypothalamic leptin expression by administration of an antibiotic. The rats with chronic transgene expression had less hypothalamic leptin expression than the group with regulated transgene expression (Zhang & Scarpace, 2006). The researchers asked whether leptin induces LR. The leptin gene was delivered to the third ventricle of the brain in lab rats through transfection. The exposed animals initially responded with less food intake but over time the effect decreased when compared to a control group. At 140 days the two groups had similar responses. This showed that while in the exposed group initially had a stronger response to central hyperleptin levels over time, the response was blunted (Zhang & Scarpace, 2006).

Circulating regulators of the leptin signaling system can also interfere with leptin reception. Yang and colleagues (2009) suggested that LR may be related to interruption of downstream signaling of STAT-3 (G. Yang et al., 2009). As discussed earlier, this mechanism of resistance would explain hypothalamic LR. It also demonstrates that resistance may be related to a transporter interaction. Banks (2008) suggests that leptin transport is enhanced by insulin, glucose and epinephrine, while inhibited by loss of ovarian function, obesity, starvation, treatment with a lipopolysaccharide and hypertriglyceridemia .

Cellular-level LR mechanisms are described in a study of mice lacking leptin (*ob/ob*) or a functional leptin receptor (*db/db*) (Barouch et al., 2003). They found that leptin was an independent determinant of LVH. In leptin-deficient mice (*ob/ob*) when leptin was replenished,

there was complete reversal of gross cardiac hypertrophy. Furthermore, in a study of leptin-deficient (*ob/ob*) and leptin-resistant (*db/db*) mice, (Raju et al., 2006) found that mice that received ciliary neurotrophic factor (CNF)—a previously undescribed signaling pathway—had reversal of gross hypertrophy. The researchers concluded that CNF was responsible for downstream leptin signaling. Finally, a study of pediatric cardiac myocytes reported that attenuation of Jak2, as well as, STAT and MAP kinases altered leptin action in these cells (Madani, De Girolamo, Munoz, Li, & Sweeney, 2006).

Extracellular circulating factors also exist that may affect leptin's action and availability according to a study reporting on the attenuating effects of C-reactive protein on leptin (Procopio, Andreozzi, & Sesti, 2007). CRP is an acute phase reactant stimulated by IL-6, which binds to a phosphocholine molecule bonded to the surface of dead or dying cells. When CRP binds to phosphocholine it activates the complement system. The purpose of this inflammatory cascade is the clearance of necrotic and apoptotic cells. In a study by Procopio and colleagues (2007), preincubated leptin was saturated in human C-reactive protein (CRP) for 30 minutes prior to treating human cells. Down-stream regulators of leptin function were impaired alluding to the likelihood that leptin function may be regulated by the body's immune system. The suggested relationship between leptin and the immune system could explain LR because the leptin receptor has been found in the IL-6 family of class 1 cytokine receptors (Tartaglia, 1997).

The process of a chronic inflammatory state in association with obesity has also been described by contemporary research, initially due to the discovery of white blood cells in atheromatous plaques. As early as the 1990's, researchers were identifying possible links between inflammatory processes and atherosclerosis (Danesh, Collins, & Peto, 1997). Additionally, coronary artery disease has been associated with elevated levels of CRP. This

discovery suggests the possibility of stronger inflammatory connection with diseases associated with obesity than previously thought.

#### *Left Ventricular Hypertrophy and Leptin*

Left ventricular hypertrophy has been linked in adult humans and animal models to a lack of leptin reception. Kartal and colleagues (2008), reported a study of adult hypertensive patients ( $N = 80$ ) and found that those with LVH had significantly higher levels of leptin than those without LVH (Kartal et al., 2008). They also reported that serum leptin levels were related to LVH independent of BMI and BP. This study generates an important question: If LVH is apparently associated with lack of leptin reception, why do adults with elevated leptin levels have LVH? For example, in some animal models where subjects are genetically modified with the inability to produce leptin, the test subjects have LVH as well (Kartal et al., 2008). Also, in a comparative study of leptin deficient (*ob/ob*) mice and control mice subjected to transverse aortic constriction, the injection of leptin into the *ob/ob* mice resulted in protection from ventricular hypertrophy by activating the NFATc4 DNA binding activity allowing increased cardioprotective markers. These results allow us to conclude that leptin possibly provides a protective mechanism against LVH in animal models (Mascareno, Beckles, Dhar-Mascareno, & Siddiqui, 2009). To answer the above question, while leptin is abundant in obesity LR prevents it from functioning as intended.

It may be considered that leptin acts an independent determinant of left ventricular hypertrophy (LVH) in leptin deficient mice as described earlier (Barouch et al., 2003). In that study, when leptin was replenished in the leptin deficient mice (*ob/ob*), there was a complete reversal of gross cardiac hypertrophy. This result has never been duplicated in human models because the prevalence of genetically leptin deficient humans is very low.

Obese children have shown evidence of LVH. Gutin and colleagues reported a study of children aged 7 to 13 years ( $N = 62$ ), reports that for LVM/height<sup>2.7</sup>, when controlling for age, partial correlation with percentage of body fat existed in the sample ( $r = .25$ ). Multiple regression analysis revealed that independent proportions of the variance were explained by gender (boys > girls), ethnicity (black > white), and percentage of fat. When controlling for age, non-normalized LVM was correlated with fat-free mass ( $r = .76$ ) and fat mass ( $r = .58$ ). Multiple regression analysis also suggested that fat-free mass explained independent proportions of the variance in LVM. Percentage of fat was correlated, when controlling for age, with greater relative wall thickness ( $r = .34$ ) and lower mid-wall fractional shortening ( $r = -.37$ ) (Gutin et al., 1998).

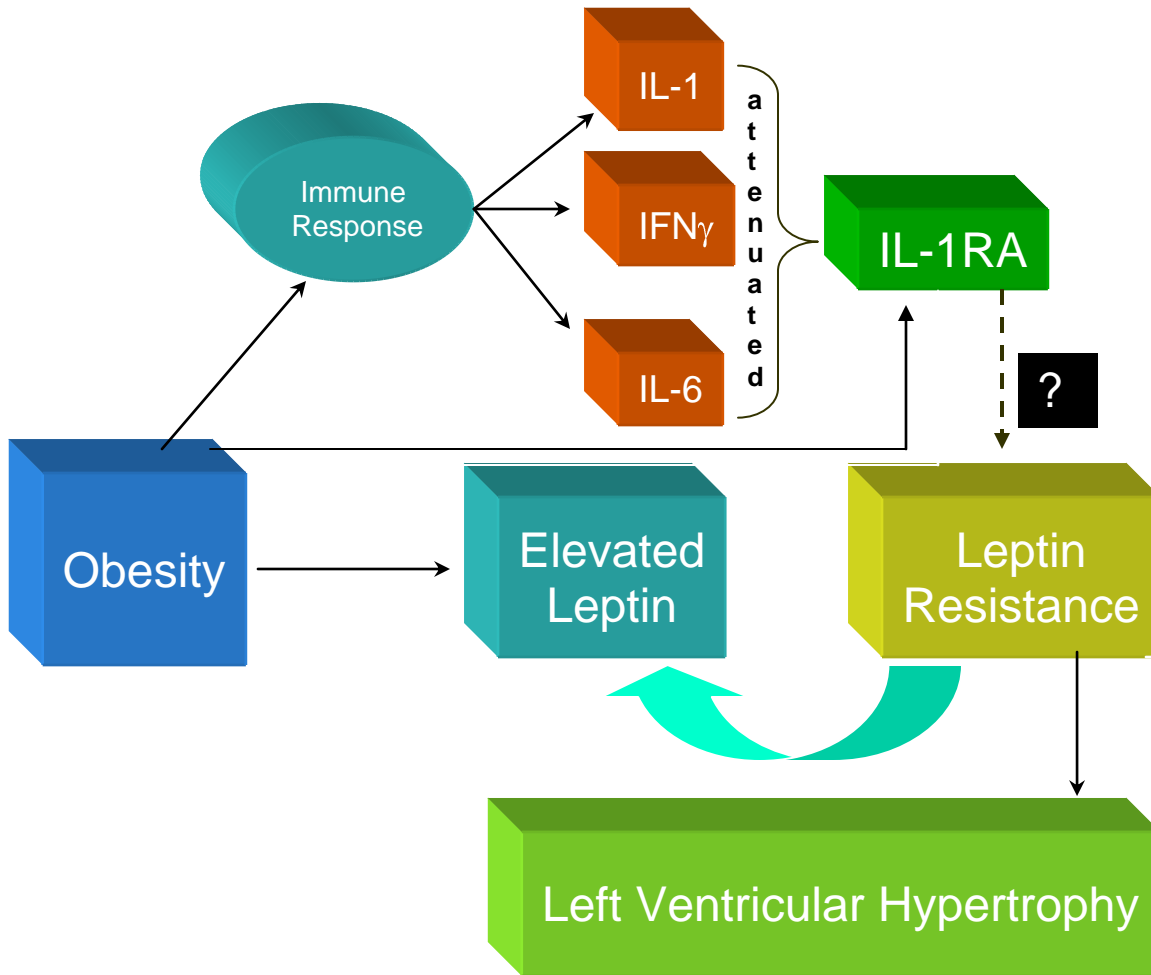
Evidence of hyperleptinemia in obese children and adolescents was reported in a study of 40 obese and lean adolescents, which concluded that mean fasting 24-hour leptin levels were significantly higher in obese boys and girls than in lean control subjects (Heptulla et al., 2001). The authors also reported no difference in leptin amplitude over a 24 hour period in obese boys or girls.

#### *IL-1RA and LVH in Obese Children and Adolescents*

Recent research suggests that the cytokines interleukin-1 (IL-1), IL-6, IL-10, and IL1-RA may be associated with obesity related diseases such as metabolic syndrome. (K.-C. Huang et al., 2003; Mendall et al., 1997; Okamoto et al., 2000). No study references were found within the body of scientific literature identifying how chronic inflammation affects heart size in obese children and adolescents. Research documenting leptin resistance linked to cytokine function in the body serves as a contextual precursor for our study of LR and LVH in obese children and adolescents (Munzberg et al., 2005; H. Yang et al., 2005). No study documentation could be found which focuses on the existence of LR attenuated by cytokines in children. The proposed



hypotheses are based on the assertion that IL-1RA plays an integral role in this process. Figure 3 demonstrates the possible processes that explain leptin resistance and LVH in obesity in obese children.



**Figure 3.** Proposed model of leptin resistance.

One premise for this study asserts that obesity causes an increase in IL-1RA. For this study, it is hypothesized that IL-1RA antagonizes cytokine pathways that activate the leptin receptor. Research has suggested strong evidence for LVH relating to LR in obese individuals (Barouch et al., 2003; Pladevall et al., 2003).

Meier and colleagues analyzed leptin and IL-1RA levels in humans (Meier et al., 2002). Three groups of individuals were evaluated. One group included 20 morbidly obese non-diabetic women, 15 of whom were evaluated before and after gastric bypass surgery. A second group included 10 non-morbidly obese women matched for weight, age and height with the group of obese women. A third group had 5 moderately obese patients ( $30 < \text{BMI} \leq 35$ ). The obese subjects from group one underwent gastric bypass surgery. The results indicated that serum IL-1RA concentrations were increased 6.5-fold in the obese patients ( $p < 0.001$ ) these subjects experienced a significant decrease in IL-1RA 6 months after bypass surgery ( $p < 0.02$ ) was also reported (p. 1185).

The db/db mouse model provides an ideal model for leptin resistance in obesity. These mice have a point mutation within the leptin receptor gene. One scientific analysis of mice that lack this functional leptin receptor (db/db) suggests administration of leptin increases IL-1RA transcription in db/db mice in a similar fashion as in the hypothalamus of normal animals (Hosoi, Okuma, & Nomura, 2002).

In a study of genetically obese rats, investigators found that administration of leptin caused a significant increase ( $p = .001$ ) in the hypothalamic levels of IL-1, compared with placebo treated rats (Luheshi, Gardner, Rushforth, Loudin, & Rothwell, 1999). Peripheral administration of leptin also induced a rise in IL-1 that was significantly higher than IL-1 levels of placebo treated animals. Injection of leptin into lean rats decreased food intake while injection of IL-1RA alone did not significantly affect food intake. The authors also discovered that co-administration of leptin and IL-1RA attenuated (by 60%) the suppression of food intake seen in leptin-treated rats, so that food intake was returned to within 70% of respective controls. These data support the concept that IL-1RA suppresses the effects of leptin in the hypothalamus.

### *Relationship Between Leptin and Chronic Inflammation*

Obesity is alternatively described as a chronic inflammatory state that may be linked to chronic disease. Chronic inflammation may be an indicator of long-term risk of the metabolic syndrome in obese teens. Research supporting this hypothesis showed increased pro-inflammatory cytokines IL-6 TNF and alpha, along with CRP levels in obese men ( $N = 198$ ) (Mendall et al., 1997). Esposito and colleagues (2003) reported a comparative study of 50 obese and 50 non-obese women showing significantly elevated levels of IL-10—a potent anti-inflammatory cytokine found in the obese women (Esposito et al., 2003). The authors stated that those with low levels of IL-10 had metabolic syndrome. Combined, these outcomes suggest that possibly anti-inflammatory cytokines offer some protection from certain chronic diseases. This supports a theoretical model for a compensatory mechanism in the body driving it toward homeostasis by antagonizing the affects of inflammatory cytokines.

The association of a chronic inflammatory state with obesity has been described (Chida, Osaka, Hashimoto, & Iwakura, 2006; Loffreda et al., 1998). One study of mice genetically engineered to suppress IL-1 and IL-6, reported well-developed obesity within 6 months (Chida, et al., 2006). The study's authors concluded that interaction between IL-6, IL-1, and IL-1RA is responsible for lipid metabolism (p. 976). Additionally, an animal-based in-vitro/in-vivo model of the effects of leptin on the secretion of cytokines suggests that leptin induces the secretion of several pro-inflammatory cytokines (Loffreda, et al., 1998).

Further evidence suggesting that leptin induces the release of inflammatory markers in the human body was reported in a study of 20 healthy, normal-weight women of reproductive age by Canavan and colleagues (Canavan, et al., 2005). Study participants were enrolled in a randomized, double-blinded, placebo-controlled trial of leptin administration. The investigators

found that the inflammatory protein, C-reactive protein (CRP), showed a statistically significant increase ( $p = .04$ ) in the treatment group compared to the control group (Canavan, et al., 2005).

Leptin's effects on IL-1 $\beta$  and IL-1RA were studied further in human endometrial tissue isolated from women undergoing hysterectomy (Gonzalez, Leary, Petrozza, & Leavis, 2003). Functional leptin receptors were analyzed for effect on secretion of IL-1 $\beta$  and IL-1RA. The authors learned that leptin, while acting through its functional receptor, triggers the secretion of IL-1 $\beta$  and IL-1RA (Gonzalez et al., 2003). A blockade of the functional leptin receptor ameliorated the effect of leptin on the IL-1 system (Gonzalez et al., 2003). Later, Pinteaux and colleagues found that leptin induced the production of IL-1 and IL-1RA during an in-vitro examination of microglial cells (Pinteaux et al., 2007). Other research has implied that leptin also stimulates production of pro-inflammatory cytokines IL-6 and tumor necrosis factor in macrophages and circulating monocytes, both in animals and humans (Zarkesh-Esfahani et al., 2001). These studies collectively prompt the assumption that leptin only plays a role in the production of pro-inflammatory cytokines.

Yet, further evidence suggests that while leptin promotes cytokine production, leptin is also stimulated by pro-inflammatory cytokines. Grunfeld and colleagues reported a study about the effects of IL-1, and TNF-alpha on Leptin mRNA expression (Grunfeld et al., 1996). Their animal model revealed that injection of these inflammatory cytokines increased leptin mRNA levels in adipose tissue, suggesting evidence of a feedback loop between leptin and inflammatory cytokines (Grunfeld et al., 1996).

A secondary hypothesis for LR was generated for our study based on the previously described agonist/antagonist relationship that exists among cytokines when obesity is present. An

increase in levels of these cytokines may directly impede the physiologic effects of leptin (Luheshi, et al., 1999).

Interferon- $\gamma$  (IFN- $\gamma$ ) is one such pro-inflammatory cytokine that has been shown to activate the Jak2 receptor and associated tyrosine kinases. Little is known about IFN- $\gamma$  and its relationship to obesity, the activation of the pathway in the arcuate nucleus, or LR. It is not known whether IFN- $\gamma$  levels are very high in anorexia and an antagonistic relationship has been demonstrated with the anti-inflammatory cytokines IL-10 and IL-1RA. A study of human subjects with anorexia, bulimia and obesity found that IFN- $\gamma$  levels became elevated in people with anorexia while remaining very low in obese people (Raymond et al., 2000). Additionally, a study of mice with induced myasthenia gravis revealed that IL-1RA suppressed IFN- $\gamma$  and IL-6 (H. Yang et al., 2005). These studies described the suppressor-activator role of pro- and anti-inflammatory cytokines in the body. The leptin receptor is found in the pro-inflammatory IL-6 family of cytokine receptors. This leads to the assumption that IL-1RA may block this receptor since it suppressed the IL-6 receptor (Luheshi et al., 1999; Raymond et al., 2000; H. Yang et al., 2005).

Further evidence that leptin is associated with inflammatory and immune system processes was reported in obese children with a homozygous frame-shift mutation in the ob gene ( $\Delta$ G133) (Farooqi et al., 2002). This mutation resulted in an unsecreted and truncated leptin protein. These children were significantly obese with profound abnormalities in T-cell function. Administration of leptin to the obese children resulted in secretion of primarily Th1 cytokines, which may in turn directly influence antibody production.

### *Interleukin-1 Receptor Antagonist (IL-1RA )*

Interleukin-1 receptor antagonist is an anti-inflammatory cytokine released to antagonize pro-inflammatory cytokines in the body by binding molecules with IL-1 receptors. In this way the body maintains homeostatic balance. Multiple sites of IL-1RA production in the body include immune cells, epithelial cells, skin keratinocytes, stromal cells and adipocytes (most important to the current study) (Perrier, Darakhshan, & Hajdуч, 2006). Action of IL-1 is inhibited by a naturally occurring receptor antagonist, IL-1RA , which blocks IL-1 from binding to its signaling receptor (Dinarello, 1997). Activation of IL-1 signaling in the brain serves as an important regulator of systemic host defense response to infection and inflammation (Horai et al. 1998; Josephs et al., 2000). This includes suppression of food intake and fever (e.g. increased thermogenesis) (Horai et al., 1998; Josephs et al., 2000).

This evidence supports the idea that as a person's weight increases, the body releases leptin to suppress appetite and increase the metabolism in its endless attempt to maintain homeostasis (Munzberg et al., 2005). It is possible that obesity causes an inflammatory response in the body: As inflammatory cytokines rise, the body releases anti-inflammatory cytokines to ameliorate the affects of the inflammatory process. Scientific evidence supporting this hypothesis includes a study of 20 morbidly obese non-diabetic people and 10 age- and sex-matched lean adults/children. The authors found a 6.5-fold increase in serum IL-1RA in the obese subjects. IL-1RA was also positively correlated in a linear manner with leptin levels (Munzberg et al. 2005). Ljung and colleagues reported that IL1-ra was correlated with leptin in both women with Rheumatoid Arthritis and their matched controls (Ljung et al., 2007). Luheshi and colleagues (1999) reported that the administration of IL-1RA into rats suppressed leptin induced expression of appetite decrease and temperature increase. Perrier and colleagues (2006) contended that IL-

1RA has both protective and deleterious effects within the body. They concluded that leptin mediates central resistance, increases adipogenesis, and elevated serum levels have been associated with DM-2 (p. 6291).

IL-1RA has an important role in the body regarding adipogenesis and appetite control. Studies have documented that IL-1RA deficient mice are lean. Many researchers suspect this results from anorexogenic affects of IL-1 going unchecked (Somm et al., 2005). Another study reported adult onset obesity in mice lacking the IL-1R1 gene combined with resistance to the anorexogenic affects of leptin (Garcia et al., 2006). Luheshi and colleagues (1999) found that administration of IL-1RA into the brain of normal rats abolishes the anorexic effect of leptin. This evidence establishes the framework for hypothesizing that when inflammatory response increases with obesity, leptin receptors might be antagonized by IL-1RA, leading to hyperleptinemia (see Figure 1).

#### *Mean Arterial Pressure (MAP) and Left Ventricular Hypertrophy (LVH)*

A number of research studies form the basis for a secondary hypothesis: LVH in obese children may be associated with BP rather than leptin resistance. As discussed earlier, LVH may be an adaptive response by the body to increased chamber wall stress. Hypertension has been found to be a significant predictor of LVM in adults and children. Several studies in adults and children have demonstrated that obesity is a predictor of LVM independent of BP (Kono et al., 1994; Paolisso et al., 2001; Rasooly & Rasooly, 1996; Yoshinaga et al., 1995). However, Feola et al., in a study of 35 adults with untreated essential hypertension and LVH found that systolic blood pressure was significantly correlated with LV-mass. According to Alpert (2001), ventricular hypertrophy in obesity is related to an increase in blood volume and cardiac output. Increased blood volume increases preload and systemic vascular resistance. Thus, the heart

stretches more during diastole and pumps harder during systole. Alpert (2001) concluded that increased cardiac output and blood volume leads to ventricular dilation and increased wall stress resulting in hypertrophy of the cardiac muscle as a compensatory mechanism.

Several studies have linked hypertension to increased LVM. Adolescents with essential hypertension ( $N = 175$ ) were reported to have cardiovascular damage that included increased LVM (Litwin et al., 2006). Blood pressure and central adiposity were independently associated with an increase in LVM in a sample of 399 obese adults (Norton et al., 2009).

Research in children shows that MAP is statistically associated with IL-1RA, insulin, and leptin levels (Mauno, Hannu, & Esko, 2008; Rosello-Lleti et al., 2009). These results allow the assumption that BP is more closely associated with LVH in children than previously thought.

A study of normotensive rats found that the infusion of IL-1 $\beta$  was associated with bradycardia and hypotension providing even stronger support for the existence of this association (Mollace et al., 2001). Recall that IL-1RA is produced abundantly by white adipose tissue (Somm et al., 2005). Adiponectin, an adipose derived secretory protein, suppresses inflammatory response by inducing the production of IL-1RA and IL-10 (Wolf et al., 2004). Adiponectin is an adipocyte-specific protein, that stimulates nitric oxide production (Pena et al., 2009). Nitric oxide binds with vascular endothelium causing vasodilatation. A study of 88 obese children found they had lower circulating levels of adiponectin (Pena et al., 2009). Another study of 59 obese adults detected a negative correlation between adiponectin and LVM (Ebinc et al., 2008). One is led to deduce that IL-1RA production in obese people may be an antagonist of another process. One explanation may be that IL-1 $\beta$ , which normally has significant anorectic effects, is quite elevated in obese people, prompting IL-1RA secretion in response as a compensatory mechanism. IL-1RA has been shown to mediate the function of inflammatory cytokines (H. Yang et al., 2005),

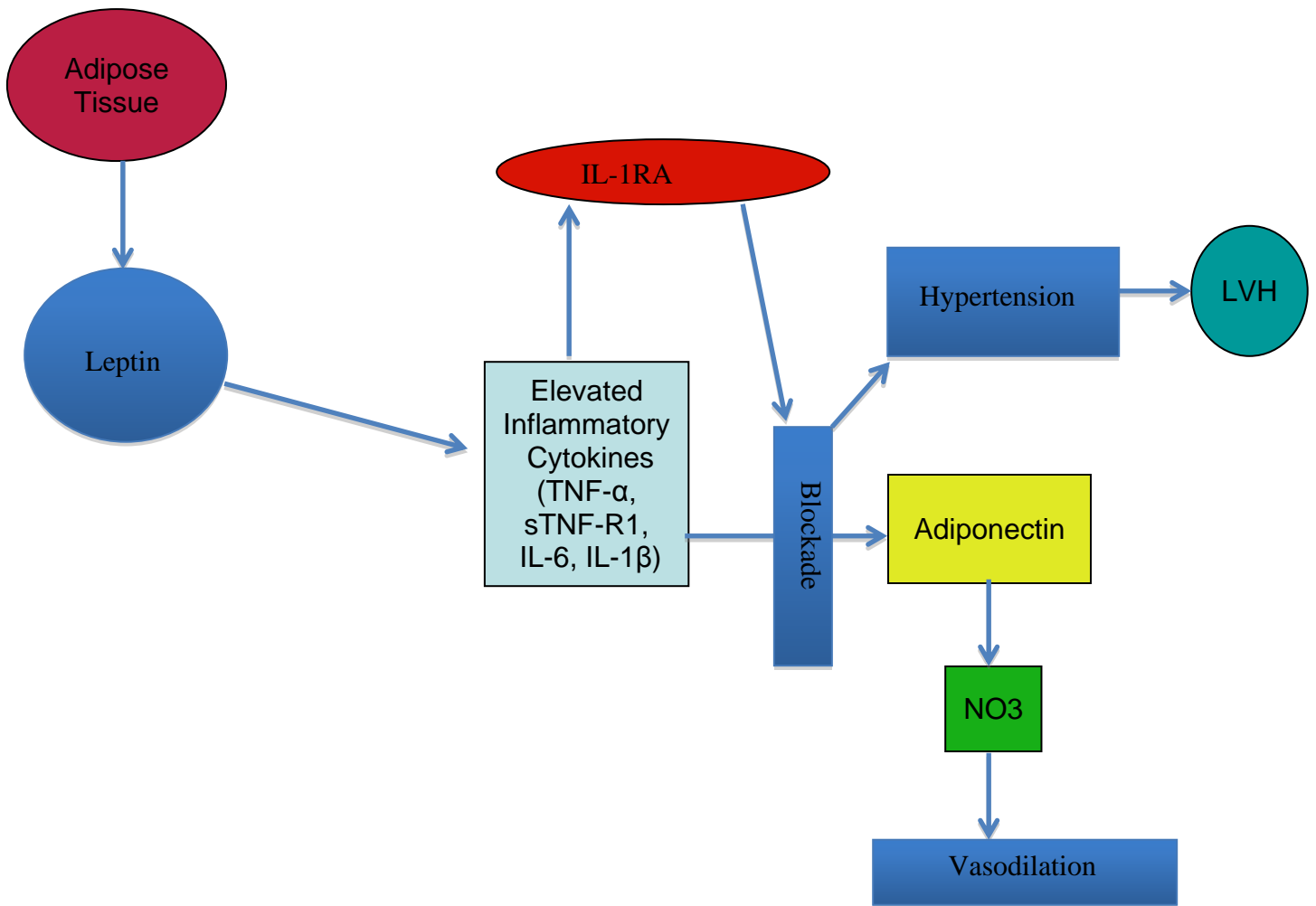


and may lead to an increase in BP by blocking the effects of adiponectin or antagonizing the hypotensive effect of IL-1 $\beta$ .

A study of 250 hypertensive patients (142 with LVH, 109 without) reports that individuals with LVH had increased cytokine levels (Rosello-Lleti et al., 2009). A prospective study of middle-aged normotensive men and women ( $N = 396, 249$ ) found that those with elevated baseline cytokine levels of IL-1 $\beta$  and IL-1RA were more likely to develop hypertension within six years (Vanhala, Kautiainen, & Kumpusalo, 2008).

Other research supports the agonist/antagonist effect theory of inflammatory cytokines and IL1-RA on BP. In a study of rabbits injected with *S. epidermidis* to induce hypotensive shock, rabbits infused with IL1-RA showed increases in MAP and systemic vascular resistance compared to non-infused specimens (Aiura, Gelfand, Burke, Thompson, & Dinarello, 1993).

Prolonged exposure to elevated arterial BP leads to an increase in LVM. Examining MAP as an independent factor in LVH in obese children and adolescents is a characteristic difference between this study of pediatric patients and previous adult studies showing that leptin was an independent determinant of LVM despite BP (Kono et al., 1994; Paolisso et al., 2001; Yoshinaga et al., 1995) Rasooly & Rasooly, 1996; Paolisso et. al, 1999). Figure 4 explains this process.



**Figure 4: Alternative Hypothesis for LVH**

## Summary

Obesity is rapidly gaining recognition as a top preventable cause of morbidity and mortality in the United States, where 21% of the U.S. annual healthcare expenditure can be attributed to the epidemic (Cawley & Meyerhoefer, 2012). Leptin is an adipokine secreted in an effort to increase metabolism and decrease appetite. Contrasting with results from animal-based models, obese humans have elevated levels of leptin, and the metabolic affects of leptin are not present in obese humans. This concept is also known as leptin resistance. Some animal models include genetically modified specimens designed without the ability to manufacture leptin. This allows researchers to explore long-term effects of leptin deficiency in the body. Leptin deficiency has been suggested as a cause of cardiac left ventricular hypertrophy. In obese adult humans the phenomenon of LVH has been associated with LR. There are no studies reported in scientific literature documenting this phenomenon in obese children and adolescents.

There are many theoretical causes for LR. Methodology for measuring LR has not gained a common consensus still. This study explored the likelihood that left ventricular hypertrophy—as a direct result of leptin resistance—is associated with the anti-inflammatory cytokine interleukin-1 receptor antagonist. No previous studies have investigated this possibility in human models. The current research also seeks to answer the question of whether or not LVH is associated with an increase in BP present in obese children and adolescents, and evaluated the possibility of an association between IL-1RA and hypertension.

### *Specific Aims*

*Aim 1: Demonstrate that leptin explains a significant proportion of the variance in left ventricular mass in obese children and adolescents.*

*Aim 2 - Demonstrate that IL-1RA is related to LV-mass and that leptin mediates this relationship in obese children and adolescents.*

*Aim 3: Demonstrate that mean arterial pressure explains a significant proportion of the variance in left ventricular mass in obese children and adolescents.*

*Aim 4: Demonstrate that IL-1RA explains a significant proportion of the variance in Mean Arterial Pressure in children and adolescents.*

## Chapter 3

### Methods

#### *Overview*

This study used a descriptive cross-sectional design consisting of two parts. First, to determine whether an association existed between IL-1RA and LVM mediated by leptin, LVM, IL-1RA, and leptin levels were measured in a sample of obese children and adolescents ( $n = 20$ ). In part, the aim was to determine if lack of reception of leptin at the cellular level (leptin resistance) leads to an increased LVM; suspect that IL1-RA inhibits leptin expression.

To determine whether or not LVH was associated with IL1-RA, a sample of 20 children from a primary-care clinic (PCC) in a suburban-metropolitan city in the western United States were evaluated. To do this leptin, IL1-RA, and insulin and LVM measured by echocardiogram were simultaneously measured. Second, to determine whether or not MAP accounts for a proportion of the variance in LVM among children, MAP and IL-1RA were compared between a group of obese children and a group of normal weight children. Finally this study tested the hypothesis that IL-1RA is significantly correlated with MAP.

#### *Study Design*

Part 1 of this study focused on children aged ten to 17 years of age with a BMI greater than or equal to the 80th percentile when accounting for age and sex. This subsample represented a group of children with obesity. Three participant-recruitment procedures were used: 1) A letter describing the study was mailed to the families of a PCC asking parents to telephone if they were interested in having their child participate in the study. Out of over 3,000 letters mailed, 103 (3.4%) respondents called the PCC to enroll. The respondents heard a summary explanation of

the study by telephone, and were invited to make an appointment for an enrollment evaluation. Of the respondents who called, 37 (1.2%) scheduled an enrollment evaluation appointment. Twelve respondents of the 37 people who participated in enrollment evaluation appointments qualified to participate and gave informed consent; 23 were not interested and 2 did not meet inclusion criteria. Of the 12 participants, one subject did not complete data collection.

Exclusion criteria prohibited children from participating who had known chronic inflammatory disease (e.g. asthma, juvenile rheumatoid arthritis, diabetes, Hashimoto's thyroid disease, collagen vascular disease) or any genetic disorder known to contribute to obesity (e.g. trisomy 21, Prater Willi syndrome, or metabolic deficiency), sleep apnea (often associated with RVH), and hypertension. Of the 37 patients who presented for an enrollment evaluation appointment, two were excluded due to asthma.

The primary care providers in the clinic were educated to the study criteria and the patients' designated primary care provider was the only person to perform a computerized or hard copy medical record review. After evaluation of the patient's history during routine clinic visits, the primary care providers described the study to the parents and, if interested in learning more about the study, provided them with the letter described in the first approved procedure, describing the research and how to contact the investigator. Once contact was made, it was determined if the subject was eligible and the study was described in further detail. The last approved method of recruitment was for direct contact of potential subjects by the researcher during the subjects regular primary care visits with him. All procedures regarding the information letter, eligibility, and contact were followed by the researcher. Nine of the 20 study participants (42.9%) were recruited through learning about the study during a routine visit to the clinic.

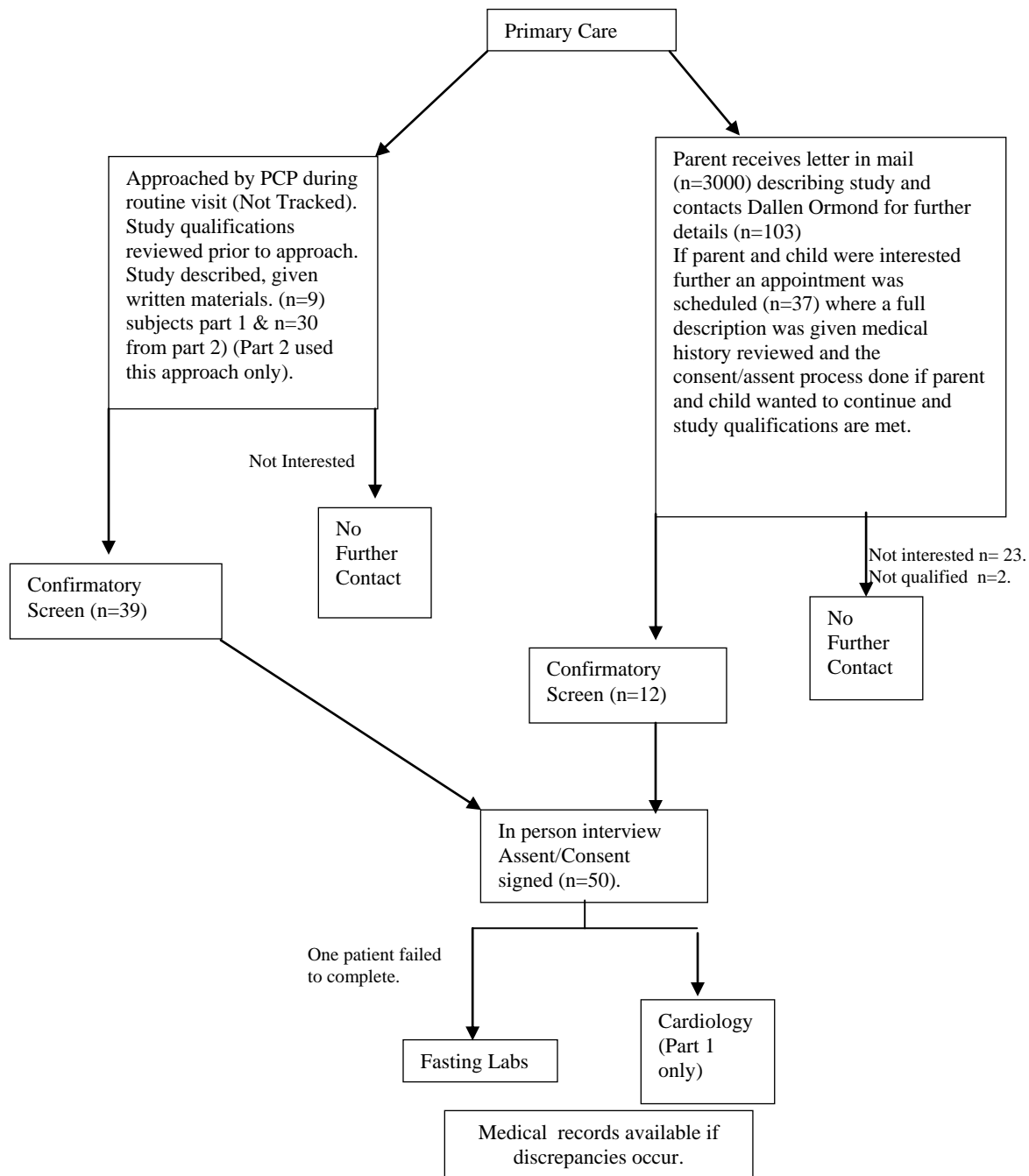
### *Procedures Part 1*

Once interest was expressed and initial contact was made with the researcher an appointment was scheduled with the potential child subject and the parent or guardian to describe the study procedures and obtain informed consent. The study's potential risks and benefits were described in detail. For those participating in the first part of the study, the research was described as a project to evaluate the thickness of the child's heart muscle in association with the hormones insulin and leptin, and a chemical in the body called interleukin 1 receptor antagonist. Those participating in the second arm of the study were told that IL-1RA was being measured to explore whether there was a correlation with mean arterial pressure. The parent/guardian and child subject were informed that the study would not help the child lose weight or determine how to help weight loss. Whereas no known harmful side-effects could result from the study, parents/guardians were informed that if any health problems were identified, particularly in the echocardiogram, the results would be immediately provided to the child's primary care provider for prompt, appropriate follow-up. Written informed consent and assent were obtained from the parent/guardian and child subject, respectively. Once written consent and assent were obtained, a review of the study criteria was conducted with the parent/guardian to confirm the child for qualification. As part of the written consent, the parent/guardian was asked for consent to review past medical records of the child in the event the medical history required clarification. The child's weight, height, and demographic information were obtained and appointments were scheduled for obtaining fasting serum samples and an echocardiogram. Each child subject received two movie tickets for participating. Figure 3 summarizes the procedures.

For part 2 of the study, Oregon Health and Science University granted IRB approval to sample 30 more patients collecting data on BMI of normal weight subjects, MAP, and fasting IL-

1RA levels. Study participants were recruited from the patient registry of a pediatric PCC by the researcher in a similar manner explained above for children recruited by the researcher personally during the subjects' regular primary care visits with him. Many patients were approached to participate during routine appointments. The majority of those who did not participate, refused due to fear over a blood draw. All procedures regarding eligibility and contact were followed by the researcher. Children aged 10 to 17 years were screened for eligibility and chronic conditions prior to inquiring about their interest in participation. For part 2 of the study, BMI categorization was not applied to the group in order to have greater variance for this measure. Individuals in this group were classified by actual weight—a continuous variable. The second part of the study built upon the results of the first and included those initial 20 subjects. Therefore the second part of the study had a sample of fifty children and adolescents to address the subsequent aims.





**Figure 5: Study Participation, consent, and qualification model.**

## *Variables*

*Demographics.* Demographic information was obtained to describe the study sample, including Race/Ethnicity (measured by self report categorized as Latino/Hispanic, Native American/Alaskan Native, Asian, Native Hawaiian or Pacific Islander, White/Non-Hispanic, and African Decent), age in years, and gender (male/female) were data collected on each child.

*Body composition.* Weight and height were measured with a standardized electronic scale and stadiometer. BMI was calculated using standardized methods:  $\text{weight [kg]} / [\text{height (m)}]^2$ .

*Mean arterial pressure (MAP).* Blood pressure was assessed using a Dynamap automated blood pressure monitor. Mean arterial pressure is approximated by:  $(2 \times \text{diastolic pressure}) + \text{systolic pressure} / 3$ .

*LV-Mass.* For the twenty subjects in the first phase of the study, LV-Mass measurements were made using echocardiographic methods validated for children with normal hearts based on the following formula:  $(\text{gm}) = 0.80 [1.04 \times [1.04 \times [(\text{IVSD} + \text{LVIDD} + \text{LVPWD})^3 - (\text{LVEDD})^3] + 0.06]$  where IVSD = intraventricular septal dimension, LVIDD = LV internal dimension during diastole, and LVPWD = LV posterior wall dimension (Daniels, Meyer, et. al., 1988). Echocardiograms were performed by a board certified pediatric echocardiographer.

*Leptin.* Leptin was measured using the Human Leptin RIA Kit (Linco Research, USA). The required sample volume was 100 microliters of serum. This sample was a fasting sample with a minimum fast of 8 hours. Specificity of human leptin was measured at 100% compared to other hormones and other mammalian leptin serum tested. Analysis was performed by the University of Utah, Government Clinical Research Center.

*Cytokines.* Fasting IL-1RA was evaluated using a Lincoplex cytokine assay. Sensitivity was 0.5-16 pg/ml, using Luminex technology. These tests are considered the standard for

measurement within the industry (Linco Research, USA). This analysis was performed at the University of Utah, Government Clinical Research Center for the subjects in the first arm of the study and the analysis for the second group of subjects was performed at Oregon Health and Sciences University using the same protocol.

### *Statistical Analysis*

All variables were evaluated for normality and outliers. The joint distributions of the study variables were evaluated for linearity in accordance with the assumptions of multiple regression analysis.

***Aim 1: Demonstrate that leptin explains a significant proportion of the variance in left ventricular mass in obese children and adolescents.***

This hypothesis was tested on the initial sample,  $n = 20$ , through regression analysis of leptin as a predictor of LV-mass. Covariates in this model included age and gender.

***Aim 2 - Demonstrate that IL-1RA is related to LV-mass and that leptin mediates this relationship in obese children and adolescents.***

To test this hypothesis, Sobel's method for testing mediation was planned (McKinnon et al., 2005). Two regression analyses were conducted on the initial sample,  $n = 20$ . In the first, IL-1RA was the independent variable and leptin was the dependent variable. In the second, leptin was the independent variable and LV-Mass was the dependent variable. The regression weight for IL-1RA from the first regression was multiplied by the regression weight for leptin from the second regression. The planned analysis was to take the product of these scores to give the mediated or indirect effect of IL-1RA through leptin on LV-mass. The following formula was to yield a z-score (McKinnon et al, 2005):  $[\alpha\beta/\text{SQRT}(\alpha^2\sigma_\beta^2+\beta^2\sigma_\alpha^2)] \rightarrow \text{Z-score}$  in which  $\alpha$  is the regression coefficient from the first regression (IL-1RA regressed on leptin),  $\beta$  is the regression

coefficient from the second regression (leptin regressed on LV-mass),  $\sigma^2_{\beta}$  is the standard error of leptin regressed on LV-mass, and  $\sigma^2_{\alpha}$  is the standard error of IL-1RA regressed on leptin.

Covariates to be included in this model were BMI, gender, and age.

***Aim 3: Demonstrate that mean arterial pressure explains a significant proportion of the variance in left ventricular mass in obese children and adolescents.***

This hypothesis was tested on the initial sample,  $n = 20$ , through regression analysis of MAP as a predictor of LV-mass. Covariates in this model included age, BMI, and gender.

***Aim 4: Demonstrate that IL-1RA explains a significant proportion of the variance in Mean Arterial Pressure in children and adolescents.***

This hypothesis was tested on the full sample,  $N = 50$ , through regression analysis of IL-1RA as a predictor of MAP. Covariates in this model included age, BMI, and gender.

## Chapter 4

### Results

In this chapter, the characteristics of the sample will be described for the initial and the total sample. The results of the first three aims, analyzed for the initial study sample of  $n = 21$ , will then be reported. The final results for the total sample,  $N = 50$ , will then be described. .

#### *Characteristics of the Sample*

The study sample was mostly Caucasian (86%), with only three Hispanic and Asian Americans, respectively, and one African American. The initial sample was comprised of 11 males and 9 females and had limited diversity; see Table 1a. The full study sample consisted of 28 males and 23 females. The mean age of those involved in the full study was 12.35 ( $sd = 2.13$ ) years old; girls were, on average, 2 years younger than boys. See Table 1b.

Table 1a *Descriptive Statistics by Gender (n = 20)*

Variable	Males $n = 9$	Females $n = 11$	Total Sample
Age (mean, $sd$ )	12.45 (2.25)	12.22 (2.10)	12.35 (2.13)
Race/Ethnicity	$n$ (%)	$n$ (%)	$n$ (%)
Caucasian	8 (40%)	9(55%)	17 (85%)
Hispanic	0	1 (5%)	1 (5%)
African American	1 (5%)	0	1 (5%)
Asian/Pacific Islander	2 (10%)	1 (5%)	3 (15%)

Table 1b *Descriptive Statistics by Gender (N = 50)*

Variable	Males <i>n</i> = 28	Females <i>n</i> = 22	Total Sample <i>N</i> = 50
Age (mean, <i>sd</i> )	13.3 (2.14 )	11.4 (2.47 )	12.35 (2.13)
Race/Ethnicity	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
Caucasian	23 (45%)	21(41%)	45 (86%)
Hispanic	2 (3.5%)	1 (2.5%)	3 (6%)
African American	1 (2.5%)	0	1 (2%)
Asian/Pacific Islander	2 (3.5%)	1 (2.5%)	3 (6%)

For this study BMI served as a continuous variable, the exception was when the subjects BMI was categorized as obese vs, non-obese. Obese BMI was categorized as  $>24.5$  for all ages. Because this was the value was equal to or exceeded the 85<sup>th</sup> percentile and is considered overweight for all ages. The BMI for the first 20 subjects ranged from 19.7 to 45.2 kg/m<sup>2</sup>, and averaged 29.68 (*sd* = 7.65) kg/m<sup>2</sup>; the average BMI for this subsample was above the 80<sup>th</sup> percentile for BMI for age. The second 30 subjects were not selected for obesity and their BMI ranged from 15.6 to 34.3 kg/m<sup>2</sup>, with an average BMI of 20.6 (*sd* = 4.29) kg/m<sup>2</sup>. For the total sample then, the average BMI was 24.13 (*sd* = 7.38) kg/m<sup>2</sup>. Mean arterial pressure (MAP) ranged from 68.7 to 102.0 for the total sample, and averaged 85.65 (*sd* = 7.32); for the initial sample of *n* = 20, MAP ranged from 79.3 to 98.0, and averaged 85.03 (*sd* = 7.65), for the second sample range 73.3-102.0, mean- . Within the total sample, the average MAP in boys was 85.26, and 85.10 in girls. Mean IL-1RA in the total sample was 552.34 (*sd* = 414.88) pg/ml; the averages by gender were comparable. The obese group with BMI  $\geq 24$ , by gender, revealed

MAP averages above the 95<sup>th</sup> percentile: 950.82 pg/ml for boys and 852.56 pg/ml for girls. See Table 2.

Table 2a BMI, MAP, and IL 1-ra values for the subgroups.

Variable	Part 1: <i>n</i> = 20	Part 2: <i>n</i> = 30
BMI (mean, <i>sd</i> )	29.68 kg/m <sup>2</sup> (7.65)	20.6 kg/m <sup>2</sup> (4.29)
MAP (mean, <i>sd</i> )	85.03 (7.32)	84.62 (7.96)
IL 1-ra (mean, <i>sd</i> )	907.15 pg/ml (455.18)	315.80 pg/ml (101.95)

Table 2b BMI, MAP, and IL 1-ra values for the total sample (*N* = 50).

Variable	Males	Females	Total
BMI (mean, <i>sd</i> )	23.93 kg/m <sup>2</sup> (7.67)	24.43 kg/m <sup>2</sup> (6.99)	24.13 kg/m <sup>2</sup> (7.38)
MAP (mean, <i>sd</i> )	84.25 (8.39)	85.58 (6.83)	85.65 (6.32)
IL 1-ra (mean, <i>sd</i> )	552.02 pg/ml (499.65 )	552.75 pg/ml (284.01)	552.34 pg/ml (414.88)

*N* = 50

Leptin, insulin, and LV-Mass were assessed in the initial sample only (*n* = 20). Levels of leptin ranged from 10 to 78.3 averaged 34.2 (*sd* = 17.9) ng/ml; males averaged 30.4 ng/ml (*sd* = 11.8) whereas girls averaged a higher leptin level at 38.9 (*sd* = 23.2) ng/ml in females. These values are in contrast to normal mean leptin values of 3.4 ng/ml +/- 3.1 ng/ml (Haqq et al., 2003). Normal insulin levels are considered to be less than 120 pmol/L. Mean insulin levels for the initial sample ranged from 2.3 to 24.5 pmo/L, and averaged 12.49 (*sd* = 7.53) pmo/L; the average was lower for study boys at 12.3 (*sd* = 6.7) pmo/L and was higher for study girls at 12.7 (*sd* = 8.87) pmol/L. LV-Mass (LVM) ranged from 27.5 to 100.1, and averaged 60.1 (*sd* = 13.9). See Table 3.

Table 3  
Leptin, Insulin, and LVM for the initial sample ( $n = 20$ ) and by gender.

Variable	Males	Females	Total
Leptin (mean, sd)	30.4 ng/ml (11.8)	38.9 ng/ml (23.2)	34.2 ng/ml (17.9)
Insulin (mean, sd)	12.3 pmol/L (6.7)	12.7 pmol/L (8.87)	12.49 pmol/L (7.53)
LVM (mean, sd) ( $n=21$ )	59.42 g/m <sup>2</sup> (18.21)	57.72 g/m <sup>2</sup> (12.33)	60.1 g/m <sup>2</sup> (13.9)

Using obese vs. non-obese as a grouping variable for differences of the variables between the two populations. BMI levels ( $\geq 24.5$  vs.  $< 24.5$ ) as the grouping variable (see Table 4),  $t$ -test was used to assess for differences between groups on leptin, insulin, and LVM; no significant differences were revealed. For the entire sample ( $N=50$ ),  $t$ -test demonstrated a significant difference in IL-1RA levels between the groups ( $t(50) = 4.386, p = 0.001$ ) but no difference was found in MAP.

Table 4  
Descriptive Statistics by BMI Group

	BMI	N	Mean	Std. Deviation
Leptin	$\geq 24.50$	16	33831.25	17791.30
	$< 24.50$	4	35850.00	20850.30
Insulin	$\geq 24.50$	16	13.07	7.11
	$< 24.50$	4	10.15	9.84
LVM	$\geq 24.50$	17	59.84	16.07
	$< 24.50$	4	53.4	12.05025
IL-1RA	$\geq 24.50$	20	821.41	497.72
	$< 24.50$	30	372.97	212.89
MAP	$\geq 24.50$	21	86.60	8.15
	$< 24.50$	30	83.630	7.22



### *Relationships between IL 1ra, Leptin, and LV-Mass*

Correlation analysis was carried out among the study variables for the initial sample; see Table 5. The level of significance was set at ( $p < .05$ ). Cohen's benchmark for effect size was also used to evaluate the strength of these correlations. Effect sizes are a family of indices that quantify the magnitude of a relationship between an independent and dependent variable (Cohen, 1988), and may be evaluated as small (.2), medium (.5) or large (.8). After data collection on the first 20 patients was completed, effect sizes were used to evaluate the first three study aims that concerned LV-Mass.

Table 5

Correlations among Study Variables for Initial Sample ( $n = 20$ )

	LVM	Leptin	IL-1RA
LVM			
Leptin	.024		
IL-1RA	.260	.217	

\* $p < .05$

The first aim of this study was to determine whether LVH in obese children and adolescents is related to leptin resistance. Since LVH is a diagnosis based on an elevated LV-Mass, LV-Mass was the dependent variable in the study. It was hypothesized that LV-Mass could increase as leptin increases, suggesting that elevated leptin levels were due to blockade of the leptin receptor by IL-1RA (leptin resistance). The small effect size associated with a correlation of  $r = .024$ ,  $p = .46$  does not support this hypothesis.

The second aim of the study was to see if leptin mediated the predictive relationships between IL-1RA and LV-Mass. This aim could not be supported because effect sizes did not reveal a significant relationship ( $r = .26, p = .13$ ). Therefore, because steps one and 2 of the mediator analysis did not show a significant correlation a regression analysis could not be completed and step 3 was not possible. This aim could not be supported.

The third aim of the study was to assess whether MAP predicted LV-Mass and explained a significant portion. However, correlation analysis revealed a small to moderate effect for MAP on LV-Mass,  $r = .36$ , that was not significant ( $p = .06$ ). Therefore, this aim was not supported.

#### *Correlations among Study Variables*

The correlations among all the study variables were reviewed in a post hoc exploratory analysis for the initial sample; see Table 6. Several correlations demonstrated a moderate to large effect size. There was a statistically significant positive correlation between body mass index and LV-Mass ( $r = .46, p = .018$ ) representing a moderate effect size. Body mass index was also positively correlated with age ( $r = .70, p = .001$ ) and mean arterial pressure ( $r = .50, p = .01$ ), in the total sample, and leptin ( $r = .56, p = .005$ ) and insulin ( $r = .57, p = .01$ ) in the smaller initial sample. These correlations represent moderate effect sizes. Mean arterial pressure yielded a large correlation with IL-1RA ( $r = .75, p = .001$ ) in the total sample and, in the initial sample, a moderate correlation with leptin ( $r = .50, p = .01$ ). Given the large correlation between BMI and age, the partial correlations were assessed when controlling for age (see Table 6b). The partial correlations remained comparable with the exception of that between BMI and MAP, which was positively strengthened,  $r = .65, p = .01$ .

Table 6a *Correlations Among Study Variables (n =20)*

	LVM	MAP	BMI	Age	Leptin	Insulin
MAP	.36					
BMI	.46*	.50*				
Age	.32	.06	.70**			
Leptin	.02	.51*	.56**	.36		
Insulin	.18	.57**	.61**	.45	.64*	
IL-1RA	.26	.75**	.29	.17	.22	.52*

\*p< .05

\*\* p<.01

Table 6b *Partial Correlations Among Study Variables Controlling for Age (n = 20; df = 17)*

	LVM	MAP	BMI	Leptin	Insulin
MAP	.36				
BMI	.34	.65**			
Leptin	-.10	.52*	.46*		
Insulin	.04	.61**	.46*	.64*	
IL-1RA	.22	.76**	.24	.17	.50**

\*p< .05

\*\* p<.01

Regression analysis with age excluded did not significantly change the  $R^2 = 0.35$  with age in the model vs. 0.38 without, ( $p = 0.31$  vs. 0.25) value for the model and the model remained statistically not significant.

#### ***Prediction of Mean Arterial Pressure***

The fourth aim of this study was to examine whether IL 1ra predicted mean arterial pressure, correlation analysis among study variables was conducted for the entire sample ( $N = 50$ ), and revealed significant relationships with small effects between MAP and age, BMI, and IL-1RA. The significant correlation between BMI and IL-1RA yielded a moderate effect size.

See Table 7a. Again, partial correlations were assessed among these variables while controlling for age; see Table 7b. With age controlled, the relationship between MAP and BMI remained comparable whereas the correlations between IL-1RA and MAP and IL-1RA and BMI were positively strengthened.

Table 7a Correlations among Study Variables ( $N = 50$ )

	MAP	Age	BMI
MAP			
Age	.30*		
BMI	.36**	.17	
IL-1RA	.38**	-.18	.59**

\* $p < .05$

\*\*  $p < .01$

Table 7b Correlations among Study Variables ( $N = 50$ ;  $df = 47$ )

	MAP	BMI
MAP		
BMI	.33**	
IL-1RA	.46**	.64***

\* $p < .05$

\*\*  $p < .01$

\*\*\*  $p < .001$

A multivariate regression analysis was subsequently performed on the entire sample to evaluate the amount of variance in mean arterial pressure explained by gender, age, IL-1RA, and body mass index. The gender, age, and BMI were entered as a block, IL-1RA was regressed with and without these variables. The variables were entered hierarchically after step 3  $R^2 = 0.19$ , with IL-1RA added to MAP then report the adjusted  $R^2$  value and accounted for a greater variance. The variables in the model accounted for 29 % of the total variance in MAP, and the model was statistically significant ( $F(4, 46) = 3.65, p < 0.02$ ). Furthermore, as shown in Table 8, IL-1RA accounted for a significant proportion of the variance in MAP; for every one standard deviation increase in IL-1RA, MAP increased 0.411. Age was also a significant predictor of MAP. An hierarchical analysis controlling for age, sex and BMI with IL-1RA regressed on MAP demonstrated that these first three variables, while still significant, accounted for far less of the variance in MAP without IL-1RA in the model ( $R^2 = .19, F(3,46) = 3.649, p = .019$ ). Individually IL-1RA accounted for 10% of the individual variance in MAP after controlling for age, sex, and BMI. Therefore, the fourth specific aim hypothesizing that IL-1RA would explain a significant amount of the variance in MAP was supported.

Table 8

*Regression Model*

Model	Unstandardized Coefficients		Standardized Coefficients	
	<i>B</i>	Std. Error	Beta	<i>p</i>
IL-1RA	.008	.003	.411	.018
BMI	.062	.173	.060	.720
Sex	.174	2.053	.011	.933
Age	1.178	.467	.367	.015

$(R^2 = .29, F(4,45) = 4.54, p = .004)$

### *Post Hoc Power Analysis*

A post hoc analysis was performed to determine the actual power of some of the findings to support the conclusions of the study. Post Hoc power analysis of the correlation analysis among the variables revealed the following:

Table 9 Post Hoc Summary

Dependent Variable	Independent Variable	Pearson's $r$	Power	$n$ needed to achieve statistical power
LV-Mass (n=20)	IL-IRA	.26	0.191	62
LV-Mass (n=20)	Leptin	.02	0.05	1002
LV-Mass (n=21)	MAP	.36	0.33	32
MAP (n=50)	IL-IRA	.75	0.81	27

As expected the results of the power analysis performed for part 1 of the study revealed the smaller sample did not have significant power. Mean arterial pressure by LV-mass did show a small to moderate effect size. This is supported by a power of 0.33 on a very small sample size. Post hoc analysis also demonstrated that the sample size for this question was very close to the least significant number.

## Chapter 5

### Discussion

This study of obese and non-obese children and adolescents has contributed five important findings to the literature of pediatric obesity, leptin resistance, and left ventricular hypertrophy. First, contrary to adult obesity literature regarding left ventricular hypertrophy, leptin resistance did not correlate with left ventricular mass in obese children and adolescents. Second, this study was unable to support a significant relationship between leptin and IL-1RA; although there was a small effect size found between the variables. Third, analysis revealed a small but suggestive relationship between mean arterial pressure with LV-mass in obese children and adolescents. Fourth, in the larger sample, children with a BMI at or greater than 24 had a significantly higher IL-1RA level compared to those with a BMI less than 24. Finally, IL-1RA explained a significant proportion of the variance in mean arterial pressure in obese and non-obese children and adolescents.

An evaluation of the variance in MAP explained by IL-1RA was performed because the strongest effect sizes were found between MAP and LVH and MAP and IL-1RA. Therefore, by evaluating the amount of variance in MAP explained by IL-1RA, a significant contribution can be made to the body of literature regarding LV-Mass leptin resistance and adipokines.

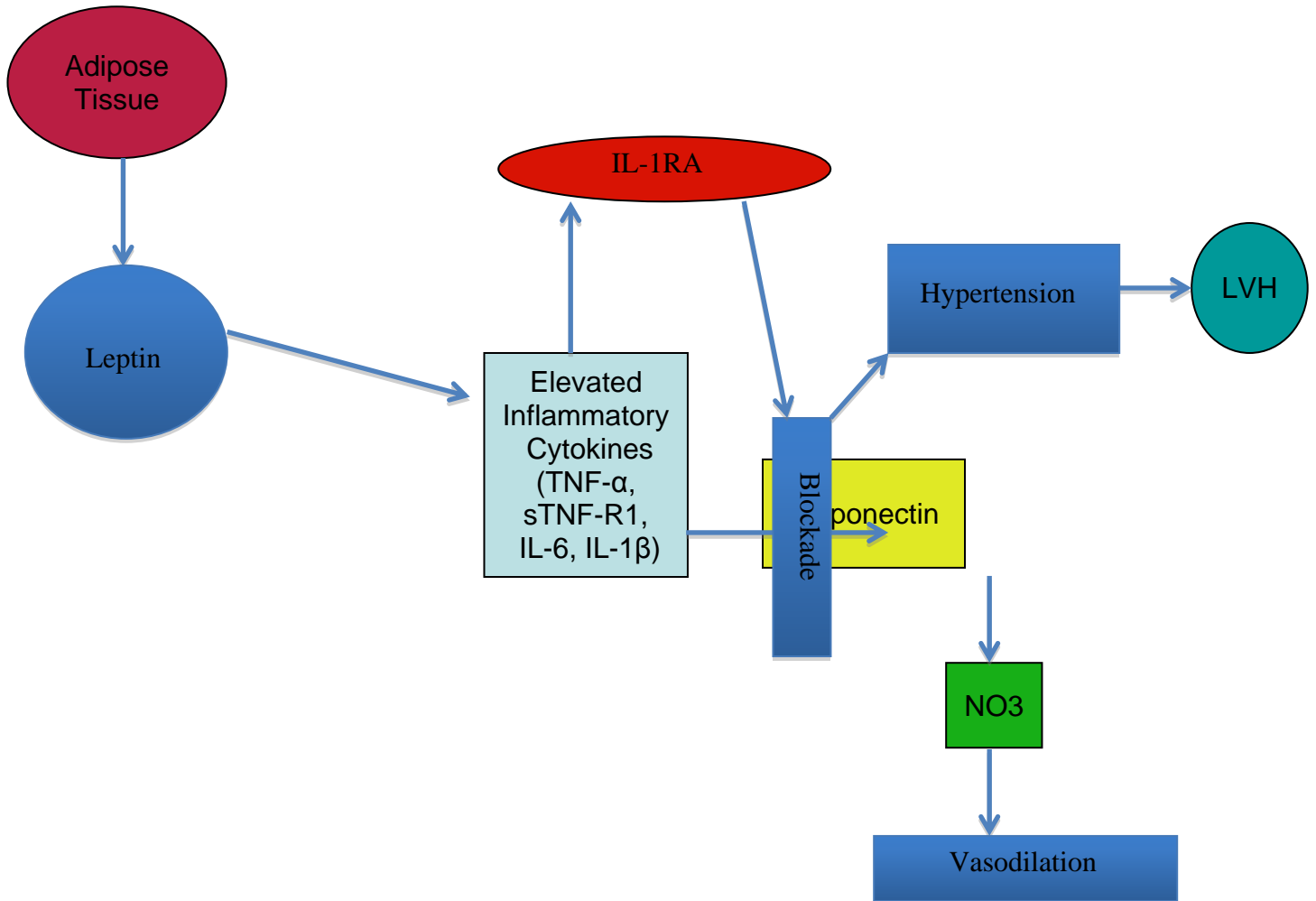
The purpose of this research was to ascertain whether leptin was associated with left ventricular mass (the outcome variable for leptin resistance), and if IL-1RA mediated this process in obese children and adolescents in order to describe a physiologic explanation for leptin resistance in humans. The findings, while lacking statistical power, revealed that the obese children as expected had markedly elevated levels of leptin mean 30.4 in males, 38.9 in females,

(normal mean leptin values of 3.4 ng/ml +/- 3.1 ng/ml (Haqq et al., 2003) but there was no association found between leptin levels and LV-mass. This finding supports the hypothesis that leptin resistance exists in obese children and adolescents, yet did not support the hypothesis that leptin resistance is associated with LV-Mass in this population. A possible explanation to this finding was reported by a Zhang and Scarpace study (2006) in which the physiologic effects of leptin were blunted with repeated hyper-exposure in rats.

Further, this research found only a small effect size between leptin and IL-1RA. This evidence contradicts previous findings (Gonzales et al., 2003). Even though this analysis lacked statistical power, it provided additional information to the body of literature regarding leptin's association with IL-1 and IL-1RA. This finding is consistent with previous research in which pre-eclamptic women were found to have significantly elevated levels of leptin and IL-1RA, but there was no correlation stated by the authors between the variables (Molvarec et al., 2011). Leptin was significantly associated with several inflammatory cytokines, including CRP and IL-6. Leptin was also associated with blood pressure in the women with pre-eclampsia. However, the investigators did not report the result of a correlation between IL-1RA and leptin, despite IL-1RA levels in the pre-eclamptic women that were almost 3 times as high as levels in the normotensive pregnant women. They also did not comment on the significance of an association between blood pressure and IL-1RA. This may have been left out of the write-up because it was not part of the hypothesis or it may show that there is more research that needs to be performed to explain these findings. While it is difficult to make conclusions based on data compared between pregnant women and obese children and adolescents, data from other research would suggest that the effects seen in the pre-eclamptic women are not unique to this population (Aiura, et al., 1993)



What the findings from this current study suggest is that the process of leptin resistance is a complex interplay of physiologic factors and principals that far exceed the explanation of a linear association. The findings do, however, seem to concur with those findings for the alternative hypothesis: IL-1RA may be one component of leptin resistance. Figure 4 illustrates a possible explanation:



**Figure 4: Alternative Hypothesis for LVH**

Leptin is an adipokine produced by adipose tissue (Li, Li, & Zhao, 2006). It affects many tissues in the body including the liver, immune system, adipose tissue, and blood vessels of the body (Li, Li, & Zhao, 2006). Leptin is also responsible for the secretion of many inflammatory cytokines, including IL-1 $\beta$ , TNF- $\alpha$ , IL-6, and adiponectin. This study does much to elucidate the interplay of the cytokine system, particularly adipokines. Leptin was previously found to be strongly associated with elevated levels of CRP, IL-6 and TNF- $\alpha$  (Molvarec et al., 2011). The findings from this research study support the findings of prior research: elevated levels of leptin and IL-1RA in obese children were demonstrated and both of these variables were significantly

associated with blood pressure in the obese subjects. Finally, IL-1RA explained a significant proportion of the variance in MAP in study children.

Based on these results, the alternative hypothesis for leptin not being directly associated with LV-Mass in obese children, whereas blood pressure is associated with LV-Mass was supported. The interplay of leptin in this process is not excluded and may very well be supported. These findings suggest a pathway of leptin resistance not previously discussed in the literature. The insight may be that leptin resistance has many facets, including central and peripheral leptin resistance. Whereas IL-1RA does not appear to directly block the leptin receptor as previously hypothesized, the down-stream effects of leptin seem to be ameliorated by IL-1RA as these findings suggest. Specifically, obese children and adolescents had an increased LV-Mass, increased levels of leptin, increased MAP, and increased IL-1RA. Although no association was found between leptin and IL-1RA, there was an association between leptin and MAP. Finally, a significant relationship between MAP and IL-1RA was found. Based on the alternative hypothesis, it may be that as adipose tissue increases leptin is released. This release of leptin may stimulate the production of inflammatory cytokines, including IL-1 $\beta$ . This induces the release of IL-1RA which has been shown to block adiponectin resulting in an increase in MAP and ultimately an increase in LV-mass. In this way, leptin resistance may be explained in many regions of the body, similar to dominos stacked on end, as the body attempts to compensate for the effect of one domino falling another falls setting off a chain reaction. IL-1RA becomes a downstream domino that causes many side effects but does not seem to directly affect the leptin receptor.

Additionally, fasting serum insulin levels were drawn on the first 20 subjects to provide additional data for future study. The subjects demonstrated a large correlation between insulin

and leptin. This information is confirmatory data of findings in the literature regarding insulin and leptin resistance and levels in obese populations. Banks (2008) suggests that leptin transport is enhanced by insulin, glucose and epinephrine, while inhibited by loss of ovarian function, obesity, starvation, treatment with a lipopolysaccharide and hypertriglyceridemia.

### *Limitations*

The first limitation of this study was that the smaller sample only included obese children and adolescents. A study sample that only included obese subjects lacks variability and limits generalization. The additional subjects had a range of weights but were not assessed on all variables. A future study could be conducted that included equal numbers of obese and non-obese subjects that would allow comparison on both laboratory and echocardiogram data.

Age proved to be a limitation in this study because it accounted for a statistically significant proportion of the variation in the samples. This was expected because BMI increases with age. In future research this could be controlled by narrowing the age range of the study population or by using z-scores and percentiles for BMI to adjust for age. Of note, it was not felt that age skewed these results because age had only a significant correlation with BMI and only among the initial obese sample.

Patient recruitment was a major limitation of this study and resulted in a sample of obese children that was so small, there was not enough statistical power to assess the laboratory data in relation to echocardiograms performed. Future studies will need to engage multiple sites in order to ensure an adequate sample size and sufficient power for multivariate regression analysis.

BMI as an assessment of obesity was found to be a limitation. Because there is so much variability in BMI outcome based on the muscle mass of the patient, a cut point measure of BMI as the definition of obesity may not be appropriate. Other anthropometric measures that are often

used include waist/hip ratio, dexameters, and body fat percentage. Each of these measures should be carefully evaluated for use in future research.

### *Future Research*

These results need to be repeated on a larger sample size to substantiate their validity. However, the results of the second phase of the research with the larger sample do hold significant statistical power and uphold current research models explaining similar processes in other populations. Further, a longitudinal design on a large pediatric population would provide data that could explain many of the proposed processes associated with leptin resistance and explain how age, time, and long term effects of life style effect leptin resistance.

To safely answer the question of leptin resistance and LVH in obese children and adolescents, a larger study needs to be performed to elucidate these observations. If, in fact, there does not seem to be a correlation between leptin and LVH in obese children and adolescents further research needs to be conducted as to why this is occurring. A large scale study performed on a large sample size of obese and non-obese children that collected data on LV-mass, MAP, IL-1RA, and leptin could evaluate if there is no association between leptin and LV-mass in children and test a mediator relationships between the variables. To further evaluate the final physiologic explanations of this study a mediator relationship between IL-1RA and adiponectin could be tested on MAP. Finally, a larger sample size is needed to determine if MAP is truly the greatest factor in LV-Mass in obese children.

### *Summary and Conclusions*

In summary the greatest information obtained from this research is the understanding that the processes involved in leptin resistance not only have multifaceted downstream physiologic outcomes, the processes that lead to leptin resistance are also multifaceted. As with every

physiologic process this research demonstrates that the processes involved in leptin resistance include age, down stream signaling, and the specific body tissue affected.

The purpose of this study was: 1) to document whether LV-mass was associated to leptin reception in obese children and adolescents, 2) to determine if IL-1RA mediates the relationship between leptin and LV-mass, 3) to determine if blood pressure explains a significant proportion of the variance in LV-mass in obese children and adolescents, and 4) to determine whether IL-1RA explains a significant proportion of the variance in mean arterial pressure in children and adolescents.

This study is the first to examine the possibility of LVH in obese children and adolescents associated with leptin reception. The original hypothesis evaluated the possibility that IL-1RA mediated the relationship between leptin reception and LV-mass. The findings of this study demonstrated that this phenomenon is likely related to several processes in the body related to obesity. What this research demonstrated was that IL-1RA probably does contribute to leptin resistance, and LVH, but not in the way hypothesized. A significant proportion of the variance in blood pressure was attributable to IL-1RA. Interestingly as new research is currently being published on this topic these findings not only concur with research that demonstrates IL-1RA is a determinant of blood pressure, but leptin also seems to be associated with blood pressure. The likely relationship is not individual but intertwined as IL-1RA appears to be a downstream player in the process that is initiated by leptin.

Additional research is needed to evaluate these interactions and if mean arterial pressure is a determinant of LV-mass that is a result of leptin resistance or if leptin resistance is still the determinant of LV-mass in adults independent of blood pressure.

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