DIFFERENCES IN WEIGHT AND FITNESS STATUS IN BOTH THE GH-IGF1 AXIS AND MARKERS OF INFLAMMATION IN ADOLESCENTS

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ABSTRACT

Peter Andrew Hosick: Differences in weight and fitness status in both the GH-IGF1 axis and markers of inflammation in adolescents
(Under the direction of Robert G. McMurray, Ph.D.)

PURPOSE: To determine if differences in the GH-IGF1 axis and TNF-α or IL-6 exist in children based on fitness and/or weight status and if expressing fitness in units per fat free mass (FFM) alters the relationship between the GH-IGF1 axis components, TNF-α, and IL-6. Additionally, the influence that inflammatory markers have on the relationship between components of the GH-IGF1 axis was assessed. **PARTICIPANTS:** Data was collected on 124 youth purposefully selected from a larger study (CHIC III, J.S. Harrell, P.I.) into four groups: normal high fit (NH), normal low fit (NL), obese high fit (OH), and obese low fit (OL). **METHODS:** Height, weight, skinfolds, body mass index (BMI), body fat percentage and predicted VO_2max (mL/min) were measured. Predicted VO_2max was scaled to VO_2max ($mL/kg_{FFM}/min$) and used to determine fitness status. Resting growth hormone (GH), total insulin-like growth factor-1 (total IGF1), free insulin-like growth factor-1(free IGF1), insulin, TNF-α, and IL-6 were obtained from fasting blood samples. **RESULTS:** GH was significantly greater in the NH group compared to the OL group. Total IGF1, free IGF1, and TNF-α were not different in any of the groups. Insulin was greater in the OH and OL groups compared to the NH and NL groups. IL-6 was elevated in the NL and OL groups compared to the NH group and in the OL group compared to the OH group. Significant correlations between GH-IGF1 axis components existed between GH and total IGF1 ($r=0.194$, $p=0.05$) and free IGF1 and total IGF1 ($r=0.607$, $p<0.001$) only. Neither TNF-α nor IL-6 contributed

to the relationship between GH and total IGF1. Only IL-6 had a significant relationship (β=-0.060, p=0.030) between free IGF1 and total IGF1 when fitness was included in the model. **CONCLUSIONS:** Fitness may reduce the obesity related GH alterations possibly involved with continued weight gain. IL-6 levels appear more affected by fitness then fat mass. When including fitness, IL-6 may influence the GH-IGF1 axis. The relationship between GH-IGF1 axis, TNF-α, or IL-6 does not change when using VO_2max scaled to total body mass versus FFM. Findings suggest that elevated aerobic fitness may limit continued weight gain.

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LIST OF ABBREVIATIONS

BMI – Body Mass index

free IGF1 – Free insulin-like growth factor 1

GH – Growth hormone

IL-6 – Interleukin 6

NH – Normal weight high fit

NL – Normal weight low fit

OH – Obese high fit

OL – Obese low fit

total IGF1 – Total insulin-like growth factor 1

TNF-α – Tumor necrosis factor α

VO_2FFM – volume of oxygen consumption per kilogram of fat free mass per minute

VO_2kg – Volume of oxygen consumption per kilogram of total body mass per minute

CHAPTER ONE

INTRODUCTION

BACKGROUND

The growth hormone – insulin-like growth factor 1 (GH-IGF1) axis is a hormonal axis that involves growth hormone (GH) release from the pituitary and stimulation of insulin-like growth factor 1 (IGF1) from the liver. This axis is involved in a number of physiological functions including muscle hypertrophy, body composition changes, bone mineral density, and cognitive functioning. The GH-IGF1 axis functions by way of a negative feedback system that is briefly outlined in the following. Stimulation of the axis begins in the hypothalamus with the release of GH releasing hormone, this in turn releases GH from the anterior pituitary in a pulsatile fashion (Hadley and Hinds, 2000). GH release is also influenced by hypothalamic somatostatin which inhibits its release (Kopchick et al., 1999). Growth hormone release occurs in a circadian pattern, being released early after the onset of sleep (Sassin et al., 1969). Exercise also stimulates the release of GH, which is likely caused by a combination of somatostatin inhibition and GH releasing hormone stimulation (Giustina et al., 1998). Once in circulation, GH has metabolic effects and more importantly, stimulates hepatic release of IGF1. Insulin-like growth factor 1 stimulates the release of somatostatin and inhibits the release of GH both in the hypothalamus and pituitary to complete the negative feedback loop (Berelowitz et al., 1981, Guistina et al., 1998). Most of IGF1 actions

are anabolic (postnatal body growth, bone growth and development, protein synthesis) and GH dependant.

Obesity causes a disruption of the GH-IGF1 axis (Bray and Bouchard, 2004). Part of the disruption can be linked to the increased insulin that often accompanies obesity. Insulin affects the negative feedback of GH by increasing hepatic tissue sensitivity to GH through increased number of cell surface GH receptors (Leung et al., 2000). The result of increased GH receptors in the liver results in a decreased amount of GH needed to stimulate IGF1 release. Elevated levels of insulin, often seen in obesity, can also affect levels of free IGF1 by inhibiting some of the binding proteins for IGF1 (Frystyk et al., 1999, Nam et al., 1997, Nyomba et al., 1997). The increased free IGF1 may potentially stimulate anabolism; therefore, increased free IGF1 may partially explain why obese individuals tend to have an elevated absolute amount of muscle mass. These mechanisms account for the disruption of the GH-IGF1 axis in obesity resulting in low GH, normal to slightly elevated total IGF1, and elevated free IGF1.

Obesity is also accompanied by increased circulation of inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) (Maachi, et al., 2004; Caballero, 2003; van Gaal et al., 2006). Both TNF-α and IL-6 have been shown to impact intracellular signaling of insulin, IGF1, and GH (Cai et al., 2005; Gao et al., 2002; Haddad et al., 2005; Kanety et al., 1995; Rui et al., 2002; Ruan et al., 2002; Stephens et al., 1997; Tan et al., 2005). However, these cytokines have also been shown to increase muscle atrophy by blocking downstream intracellular IGF1 signaling (Aguirre et al., 2000; Gao et al., 2002; Rui et al., 2002). Increased muscle mass, possibly due to elevated free IGF1, with increased

circulating inflammatory cytokines theoretically increasing muscle atrophy, illustrates the complexity of the GH-IGF1 axis disruption in obesity.

Physical fitness in obese individuals can return the GH-IGF1 axis to a more normal state, by influencing insulin sensitivity and decreasing inflammatory cytokines. Several intervention studies have shown that increasing physical fitness increases insulin sensitivity of obese children (Bell et al., 2007; Chang et al., 2008; Landt et al., 1985; Nassis et al., 2005), adults (Brown et al., 1997; Christ-Roberts et al., 2004; Ratzmann et al., 1981; Ross et al., 2004), and healthy sedentary individuals (Ross et al., 2004: Lehmann et al., 1997; Soman et al., 1979). When resting insulin levels decrease, the amount of IGF1 binding proteins increases, leading to lower levels of free IGF1. The lower level of free IGF1 has less of an inhibition on GH release (Berelowitz et al., 1981, Giustina et al., 1998). Increased pulsatile release of GH in effect, allows GH to increase it's involvement in those physiological processes that are not stimulated through IGF1, such as increased use of fatty acid as an energy source.

PURPOSE OF THE STUDY

In the US, the most recent estimates show that 18.7% of all children age 6-19 are obese (defined as $\geq 95^{th}$ percentile) and 13.3% are $\geq 97^{th}$ percentile (Ogden, 2010). Additionally, the most extreme BMI groups for boys are continuing to increase (Ogden, 2010). This suggests that while the number of children who are overweight or obese may not be increasing, those that are overweight or obese are continuing to gain weight, particularly boys age 6-19 years. Therefore, research exploring factors that potentially contribute to the problem of continued weight gain is needed. Hormonal alterations of the GH-IGF1 axis in obesity may play a role in perpetuating the problem once weight gain begins. Further,

research is lacking on the effect of physical fitness and inflammation on the GH-IGF1 axis in obese children. Increased knowledge of how physical fitness affects the GH-IGF1 axis in obese adolescents could be used to better determine risk for continued weight gain leading to chronic health problems in this population. Therefore, the purposes of this investigation were to: 1) determine if differences in the GH-IGF1 axis of adolescents exist between high and low fitness and obese and normal weight adolescents, 2) determine if differences in TNF-α and IL-6 of adolescents exist between high and low fitness and obese and normal weight adolescents, and 3) examine the influence of inflammatory markers (TNF-α and IL-6) on associations between components of the GH-IGF1 axis of adolescents from a wide range of weight and fitness status. These purposes will be evaluated comparing the traditional unit of fitness (VO_2kg) and a unit that does not include fat mass when calculating fitness (VO_2FFM).

OPERATIONAL DEFINITIONS

Normal weight: a BMI >5^{th} and <85^{th} percentile for the child's age and sex.

Obesity: BMI $\geq$ 95^{th} percentile for the child's age and sex.

VO_2/kg_{FFM}/min: maximal aerobic power, expressed in mL of oxygen consumed per kilogram of fat free mass per minute.

High fitness: possessing a predicted VO_2/kg_{FFM}/min at or above the 66^{th} percentile for their age and gender.

Low fitness: possessing a predicted VO_2/kg_{FFM}/min below the 33^{th} percentile for their age and gender.

OH group: participants who are obese and possess high fitness based on the definitions for obesity and high fitness provided.

OL group: participants who are obese and possess low fitness based on the definitions for obesity and low fitness provided.

NH group: participants who are normal weight and possess high fitness based on the definitions for normal weight and high fitness provided.

NL group: participants who are normal weight and possess low fitness based on the definitions for normal weight and high fitness provided.

RESEARCH AIMS AND HYPOTHESES

The primary purpose of the proposed project was to determine if differences in the GH-IGF1 axis and inflammation exist in children based on fitness and weight status. To accomplish this, three major aims were developed. The first compared the components of the GH-IGF1 axis in obese and normal weight children of high and low fitness level. This aim was the focus of manuscript #1. The second research aim was to determine if differences in TNF-α and IL-6 exist in normal and overweight children who possess either high or low fitness; this aim was the focus of the second manuscript from this project. Finally aim three, the focal point of manuscript #3, assessed the influence that inflammatory markers (TNF-α and IL-6) have on the relationship between components of the GH-IGF1 axis with groups combined.

Aim 1: To determine if there are differences in components of the GH-IGF1 axis in children that are 1) normal weight and have high fitness, 2) normal weight and have low fitness, 3) obese and have high fitness, and 4) obese and have low fitness.

Hypothesis 1a. The OL group will have significantly lower morning fasted GH compared to the NH, NL and OH groups, which will all be similar. The reduced levels of GH in the OL are due to the extra adipose tissue coupled with low fitness

level, therefore, likely elevated insulin levels. Increased levels of insulin increase the number and production of GH receptors resulting in less GH required to stimulate IGF1 (Lueng et al., 2000).

Hypothesis 1b. Morning fasted total IGF1 will not be different between any of the groups. The obese children may have an elevated number on GH receptors in the liver allowing for maintenance of normal total IGF1 level in spite of reduced GH (Lueng et al., 2000).

Hypothesis 1c. The morning fasted free IGF1 will be greater in the OL group compared to the OH, NH and NL groups because the OL is expected to have elevated levels of insulin which inhibits several IGF1 binding proteins (Nyomba et al., 1997). The OH, NH, and NL groups will not be different from one another because the OH groups increased level of fitness that maintains insulin sensitivity (McMurray et al., 2000; Nassis et al., 2005).

Aim 2: To determine if there are differences in TNF-α and IL-6 in children that are 1) normal weight and have high fitness, 2) normal weight and have low fitness, 3) obese and have high fitness, and 4) obese a have low fitness.

Hypothesis 2a. Resting IL-6 will be elevated in the OL group compared to the OH group because of the inverse relationship between fitness and resting IL-6 (Kullo et al., 2007). Resting IL-6 will be similar in NH and NL groups even though the NL has reduced fitness because the major site of IL-6 production is infiltrated macrophages of adipose tissue (Shoelson et al., 2006; Eder et al., 2009). Because the NH and NL

groups have similar levels of adipose tissue IL-6 production in a resting state will be similar.

Hypothesis 2b. Resting IL-6 will be elevated in the OH group compared to the NH and NL groups because of the elevated level of adipose tissue in the OH (Shoelson et al., 2006).

Hypothesis 2c. Resting TNF-α will be elevated in the OL group compared to the OH group because of the inverse relationship between fitness and resting TNF-α (Beavers et al., 2010; Hamer et al., 2007; Thomas and Williams, 2008). Resting TNF-α will be similar in NH and NL groups even though the NL has reduced fitness. These groups will have similar TNF-α level because the major site of TNF-α production is infiltrated macrophages of adipose tissue and the NH and NL groups have similar levels of adipose tissue TNF-α production in a resting state will be similar (Hotamisligil et al., 1993; Weisberg et al., 2003).

Hypothesis 2d. Resting TNF-α will be elevated in the OH group compared to the NH and NL groups because of the elevated level of adipose tissue in the OH (Dedoussis et al., 2010; Halle et al., 2004; Hotamisligil et al., 1993).

Aim 3: To determine if inflammatory markers (TNF-α and IL-6) influence the association between GH, total IGF1, and free IGF1 in children from a wide range of fitness and weight status.

Hypothesis 3a. In the overall sample, TNF-α will influence the association between GH, total IGF1, and free IGF1. The associations will be modified by obesity and fitness.

Hypothesis 3b. In the overall sample, IL-6 will influence the association between GH, total IGF1, free IGF1. The associations will be modified by obesity and fitness.

SIGNIFICANCE

No previous work in this area has also accounted for the level of fitness of the subjects as a potential influence upon the relationship of obesity on the GH-IGF1 axis. Furthermore, no previous literature has removed fat mass when calculating fitness. This unique approach allows for comparison of obese and non-obese subjects based on fitness. Because of this, the proposed project will be able to answer several important questions that will provide further information and understanding of the interaction between fitness level, obesity status, the GH-IGF1 axis, and obesity related inflammation. Research is needed to understand the effect of obesity on free and total IGF1 as will be done in aim one. Also, particularly in children, little is known about the effect fitness has on GH or IGF1. Little work has looked at the GH-IGF1 axis in obese adolescents as it relates to inflammation. This project explored how inflammation may be altered by fitness and obesity (aim two) and how inflammation relates to the GH-IGF1 axis (aim three). With this information research can begin to explore the effects and potential positive outcomes of improved fitness in obese adolescents.

CHAPTER TWO

REVIEW OF LITERATURE

INTRODUCTION

Growth hormone (GH) and insulin like growth factor-1 (IGF1) are involved in a number of physiological functions including muscle development and hypertrophy, body composition, bone mineral density, and cognitive functioning (Alexopoulou et al., 2010; de Bie et al., 2010). These two hormones can have independent effects, but also work in unison to elicit effects as the GH-IGF1 axis. Obesity is known to modify the GH-IGF1 axis, but little is known in children as to the interactions of obesity, fitness, and varying cytokines on the GH-IGF1 axis. The goal of this project is to better characterize the influence of fitness and inflammation on the GH-IGF1 axis in obese children; therefore, the following literature review places emphasis on what is presently known about the GH-IGF1 axis as it relates to obesity and fitness. In addition, effects of training as related to fitness are discussed. The review will begin with a discussion of the individual components of the axis; GH, IGF1, and insulin. Next, is a discussion of adipose derived inflammatory markers (TNF-α and IL-6) that can cause alterations of the GH-IGF1 axis, as they pertain to obesity and fitness. Finally, known interactions between components of the GH-IGF1 axis, insulin, and inflammatory markers are highlighted.

COMPONENTS OF THE GH-IGF1 AXIS

Growth Hormone

Growth hormone is a protein-based polypeptide hormone containing 191 amino acids, that is produced and released by somatotropic cells of the anterior pituitary (Kopchick et al., 1999). Growth hormone is regulated by two hormones of the hypothalamus: growth hormone releasing hormone (GHRH) which stimulates the release of GH, and somatostatin (SST) which inhibits GH release (Figure 1). Growth hormone circulates through the blood in two states: free or bound. When GH circulates in the bound state it is attached to one of two GH binding proteins (GHBP). Two different GHBP have been discovered: high and low-affinity, (Fisker, 2006). The high affinity form of the binding protein is generated by proteolysis of the GH receptor (GHR), which is produced when the extra-cellular portion of the GHR is shed (Zhang et al., 2000). One enzyme linked to GHR shedding and production of high affinity GHBP is tumor necrosis factor-α converting enzyme (TACE) (Zhang et al., 2000; Schantl et al., 2004). In plasma, high-affinity GHBP forms a complex with GH, creating a GH reservoir (Baumann et al., 1988) that protects GH from degradation and prolongs half-life (Baumann et al. 1987). High-affinity GHBP may also act as a modulator of GH action at the tissue level by competing with GHR for ligand binding (Mannor et al., 1991). The other GHBP, or low affinity form, is estimated to carry between 5–20% of circulating GH. The precise nature or function of the low affinity GHBP is unknown (Baumann and Shaw, 1990; Baumann et al., 1989). However, the low affinity form has been demonstrated to carry other growth factors, such as platelet-derived growth factor, transforming growth factor-fl, basic fibroblast growth factor, and nerve growth factor (Borth, 1992). Regulation of circulating GH is likely the main function for both GHBPs; however,

this has not been definitively proven. In general, binding GH to its GHBP alters the strength of the effect of GH. Free GH is the physiologically active state of the hormone, but the amount of free GH is in part determined by amount of GHBP present. Thus, regulation of GHBP is an important aspect in the overall function the GH-IGF1 axis.

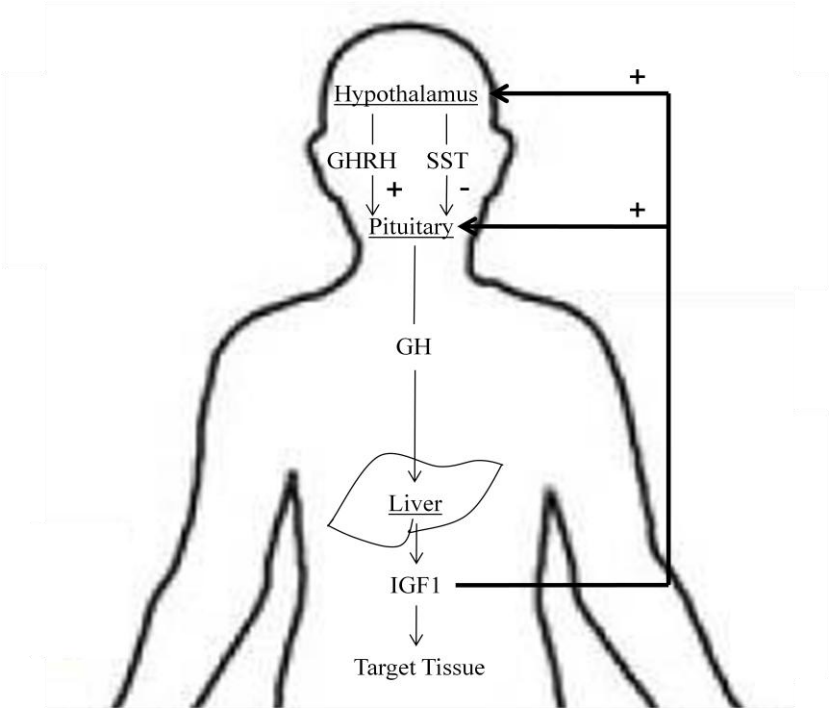

Figure 1. Model for basic control of the GH-IGF1 axis (adapted from Hadley, 2000)

GH actions are mediated through the GH receptor (GHR) which has intra-, trans-, and extra-cellular components (Fisker, 2006; Kopchick et al., 1999). Activation occurs when dimerization of one GH molecule binding to two GH receptors (Cunningham et al., 1991). Dimerization appears to be required for propagation of the GH signal and transduction of the pathways involved (Fuh et al., 1992; Xu et al., 1991). Activation of Janus kinases (JAKs) are the initial step in most GH initiated effects; additional pathways linked to GH activation include the signal transducer and activator of transcription (STAT) pathway, the insulin

receptor substrate (IRS) pathway, and the protein kinase C pathway (Kopchick et al. 1999). The GHR itself is ubiquitously expressed and has direct effects in several tissues including bone, muscle, adipose, and liver. One of the effects of GH in the liver is to increase growth factors known as insulin-like growth factors (IGFs), specifically IGF1 which will be discussed further in the following section.

Insulin-like growth factor 1

IGF1 is one of two main insulin-like growth factors (the other being IGF2) and is structurally similar and functionally related to insulin. This review will focus on IGF1 because of the independent actions between IGF1 and IGF2 and the closer relationship between IGF1 and GH. IGF1 is single chain polypeptide consisting of 70 amino acids and released principally from hepatic tissue. Synthesis of IGF1 is stimulated by GH; however, insulin is indirectly involved by enhancing hepatocyte sensitivity to GH (Clemmons, 2007; Leung et al., 2000). IGF1 is also involved in the regulation of circulating GH both directly, by inhibiting GH release at the pituitary, and indirectly, by stimulating somatostatin release (Figure 1). IGF1 is responsible for muscle development and hypertrophy and its release is regulated mainly by GH; thus, IGF1 is the major link between GH and both muscle development and hypertrophy (Adamo, 1995).

IGF1, like GH, is found in free and bound forms. The free form is the most biologically active. Also like GH, IGF1's effects can be altered by the presence of binding proteins which normally bind up to 99% of all circulating IGF1 (Frystyk, 2004). These binding proteins can increase or decrease IGF1 action in cells by binding or releasing IGF1 (Denley et al., 2005). There are six different IGF1 binding proteins (IGFBP) named IGFBP-1 through -6; each ~30 kDa binding protein has only slight differences along the linkage

regions of the individual binding proteins (Denley et al., 2005). The majority (75-80%) of circulating IGF1 is bound to IGFBP-3, whose major function is to stabilize IGF1 concentrations by increasing the half-life of the IGF1 molecule (Clemmons, 2001; Denley et al., 2005; Frystyk, 2004). The increased half-life of the IGF1-IGFBP-3 complex is accomplished mainly by adding acid labile subunit (ALS) to the complex (Frystyk, 2010). IGFBP-2 and -1 are the second and third most abundant BP's and have a significantly lower half-life then the IGF1-IGFBP-3-ALS complex. Additionally, IGFBP-1, and to a lesser degrees IGFBP-2, have been shown to be inhibited by insulin (Nyomba et al., 1997). The inhibition of IGFBP-1 and -2 by insulin appears to increase circulating levels of free IGF1 (Nyomba et al., 1997). Finally, IFGBP-4, 5, 6 circulate in very small concentrations and are believed to serve as a "reserve" for IGF1 (Clemmons, 2007). Because such a large portion of IGF1 is bound in plasma, changes in free IGF1 give a better description of the meaningful changes in IGF1.

The structural similarity between IGF1, IGF2, and insulin also extend to their receptors as all three can bind to each others receptor with varying levels of potency. Further, common pathways include the phosphorylation of intracellular tyrosine kinase, induction of IRS1 (Laviola et al., 2007; Myers et al., 1993; O'Connor, 1997) and phosphatidylinositol 3-kinase (PI3K) pathways (Clemmons, 2007; Frystyk, 2010; LeRoith and Yakar, 2007). Ultimately there is convergence upon two major signaling pathways, those being downstream mitogen-activated protein kinase/extracellular singal-regulated kinase (MAPK/ERK) and protein kinase B (AKT/PKB) (Himpe and Kooijman, 2009; Klammt et al., 2008; Laviola et al., 2007). While certain aspects of the IGF1 and insulin pathways are similar, the complete pathways are not the same (Klammt et al., 2008). The

differences between them are believed to explain the metabolic and growth promoting differences between IGF1 and insulin.

Insulin

Insulin is a polypeptide hormone consisting of two chains, a 21 amino acid A chain and a 30 amino acid B chain linked together by two disulfide bonds. The active circulating form of insulin is synthesized from a proinsulin molecule that includes a connecting peptide between the A and B chains of insulin. Proinsulin is derived from preproinsulin which includes an extra 23 amino acid sequence on the C-terminal of the proinsulin molecule.

Insulin is released in response to elevated levels of glucose from the β cells of pancreatic islets and is important for glucose uptake at tissues such as the liver, muscle and adipose tissue. Insulin effects are stimulated through the insulin receptor. The insulin receptor consists of two α and two β subunits which, when insulin binds them, cause a conformation change leading to activation of tyrosine kinase. Activated tyrosine kinase leads to the phosphorylation of multiple intracellular proteins including insulin receptor substrate 1 and 2, phosphatidylinositol-3. The activation pathway diverges with several major metabolic actions initiated by insulin binding.

Insulin is not one of the hormones that fall directly in the GH-IGF1 axis. However, it is included as one of the components of the axis because it can influence and alter circulating levels of both GH and IGF1; the two central hormones of the axis. Insulin can impact GH levels by increasing the number of cell surface GH receptors in the liver (Lueng et al., 2000) resulting in less GH required to maintain normal total IGF-1 levels. IGF-1 is altered by insulin through insulin's inhibition of several IGF1 binding proteins. Decreased IGF1 binding proteins have been shown to increase levels of free IGF1 (Nyomba et al., 1997).

Insulin is included as one of the components of the GH-IGF1 axis because of insulin's influence on the axis and because these effects can become significant in individuals with elevated levels of insulin, as commonly seen in obese individuals.

GH-IGF1 AXIS COMPONENT FUNCTIONS

Growth Hormone

Growth hormone, stimulated by acute exercise (Kanaley et al., 1997), fasting (Norrelund et al., 2004) and deep sleep (Van Cauter et al., 2004), causes release of hepatic glucose, use of fats as a fuel source, and synthesis of body proteins (Leroith and Yakar, 2007; Vijayakumar et al., 2010). To understand GH's effects on metabolism an appreciation for the evolutionary relationship between insulin, IGF1, and GH is beneficial. In primitive organisms without a pituitary gland the olfactory region of the brain produced the structurally similar hormones insulin and IGF1 as one in response to food intake (Clemmons, 2004; Tatar et al., 2003). This organization links food and carbohydrate intake with growth. In higher species, with a pituitary gland, an additional level of control is added by using GH to control IGF1 concentration. Furthermore, GH has functions that provide energy when it is not readily available in the form of glucose or glycogen (Clemmons, 2004). GH inhibits insulin actions by increasing lipolysis, glycogenolysis, gluconeogenesis, and fatty acid oxidation to provide available substrate in between meals (Clemmons, 2004; Davidson, 1987; Dieguez et al., 2000; Holt et al., 2003; Kophcick et al., 1999; Lee et al., 1999). The inhibition of insulin action by GH can be seen during long term GH supplementation in healthy adults. Munzer et al. (2009) showed that 6 months of GH injections, 3 times per week, resulted in increased circulating triglycerides and insulin area under the curve (AUC) for both male and female subjects. The same study showed females had increased fasting insulin, whereas males

increased glucose AUC and decreased both glucose tolerance and insulin sensitivity. Thus, chronically elevated GH, above normal levels, can have detrimental effects on insulin's ability to handle carbohydrate. Therefore, GH is considered an insulin antagonist.

Conversely, insulin treatment in human hepatic cells has shown to increase the number of intracellular and cell surface GH receptors, as well as, GH binding (Leung et al., 2000). Hence, when insulin is high GH can easily bind its receptor and increase IGF1, which is then involved with growth of tissue. Consequently, when glucose is being brought into the cell by insulin, GH will increase IGF1 in the cell and IGF1 can use the glucose to increase growth. Therefore, both the stimulus and fuel to power tissue growth are present at the same time.

Insulin-like growth factor 1

Insulin-like growth factor 1, stimulated via GH, mediates the growth stimulating effects associated with GH. IGF1 is involved in signaling mitogenesis leading to proliferation of cells, protein levels, DNA synthesis, uptake of amino acids and glucose, as well as a suppression of proteolysis (Laviola et al., 2007). Baker et al. (1993) have shown through growth kinetics that IGF1 is essential for both fetal and postnatal body growth. Mouse embryos exhibit delayed bone development when carrying null mutations of the genes encoding IGF1. Once born, these mice continue to grow at a slower rate compared to wild-type animals (Baker et al., 1993). An essential endocrine role of IGF1 in postnatal growth has also been confirmed using the Cre/loxP system to create IGF1 deficient mice (Liu and LeRoith, 1999). IGF1 deficient mice had an adult body weight approximately one third and body length about two thirds that of their wild-type counterparts. Additionally, injection of GH during development failed to stimulate growth (Liu and LeRoith, 1999). These findings

demonstrate the importance of IGF1 in growth and development during fetal and post natal development.

IGF1 is involved with growth and development, therefore it is not surprising that it can impact macronutrient metabolism. Research has exposed important roles for the GH-IGF1 axis in control of macronutrient metabolism. IGF1 suppresses hepatic gluconeogenesis and increases whole body glucose uptake (Ranke, 2005; Rossetti et al., 1991). Because of this effect, recombinant human IGF1 has been used to treat type I and II diabetes with some success (Ranke, 2005). IGF1 has an anti-lipolytic effect in adipose tissue, despite the fact that mature adipocytes have very few IGF1 receptors. This effect occurs because IGF1 can weakly bind the insulin receptor which inhibits fatty acid breakdown (Leroith and Yakar; 2007; Zizola et al., 2002). Also, any GH-induced increase in blood triglyceraldehyde level is not believed to be mediated by IGF1 because mature adipocytes do not have many IGF1 receptors (Zizola et al., 2002). Finally, the effects of IGF1 and GH on protein metabolism are a prime example of the GH-IGF1 axis in practice. The effects of GH, being mediated through IGF1 (Mauras and Haymond, 2005), increase protein synthesis via the IGF1 receptor and suppress proteolysis via the insulin receptor, when IGF1 concentrations are high (Turkaji et al., 1992).

Insulin

Insulin's main metabolic function is to reduce and store blood glucose. Elevated blood glucose levels are a potent stimulator for insulin release. Insulin action occurs through activation of multiple intracellular pathways that begins with insulin binding to its cell surface receptors. The insulin signaling pathway is complex and not completely understood, but leads to leads to a number of actions inside the cell. These actions include: 1) increased

glucose uptake by muscle and adipose tissue via translocation of GLUT 4 to the cell surface, 2) reduced hepatic glucose production by inhibiting glycogen breakdown and gluconeogenesis, 3) stimulate glycogen synthesis in the liver and muscle, 4) stimulate fatty acid and triglyceride production and storage, and 5) stimulate amino acid uptake and protein synthesis (Holt et al., 2003; Krook et al., 2004; Whiteman et al., 2002).

FACTORS INFLUENCING COMPONENTS OF THE GH-IGF1 AXIS

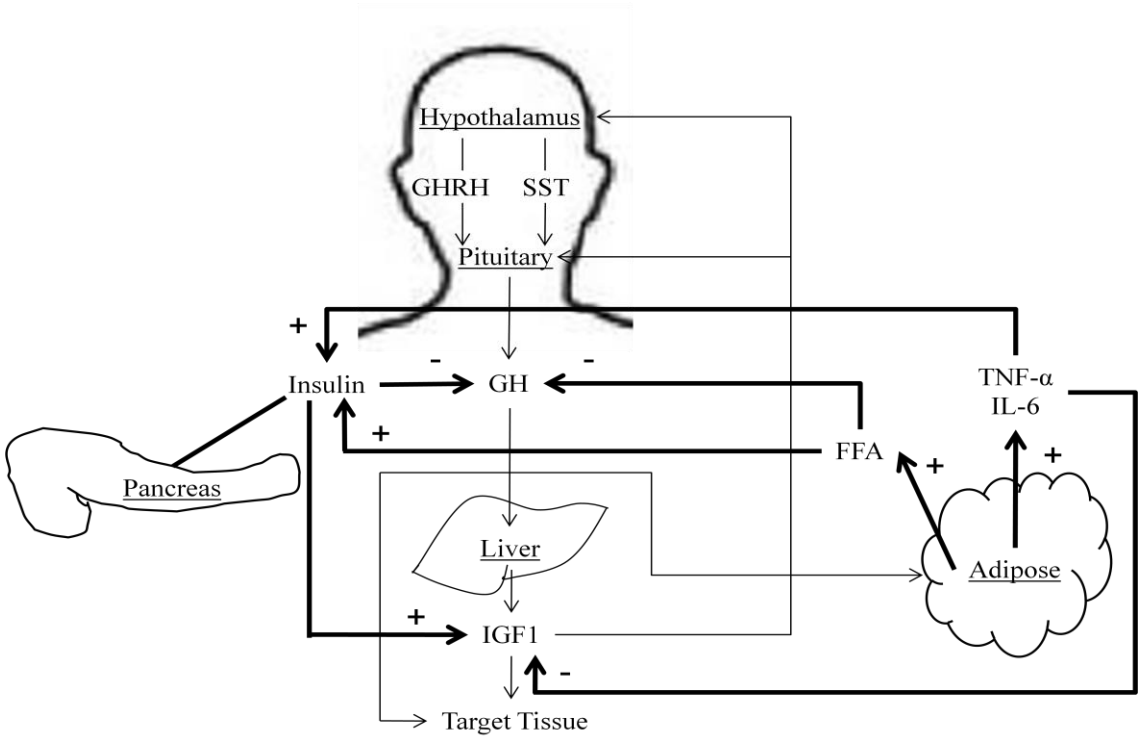

Figure 2. Model of alterations of the GH-IGF1 axis in obesity

Growth Hormone

Growth hormone (GH) is released in a pulsatile fashion causing wide and rapid fluctuations in circulating concentrations. Increased levels of circulating GH are found during deep short wave sleep (Obal and Krueger, 2004; Steiger, 2003; Van Cauter et al., 1998), hypoglycemia (Roth et al., 1964), acute fasting (Glick et al., 1965), increased core temperature (Christenson et al., 1984), and acute resistance and endurance exercise (Pritzlaff

et al., 1999; Nindl et al., 2001; Weltman et al., 1992; Wideman et al., 2004). Conversely, many obese adults and children have lower levels of both resting GH (Iranmanesh et al. 1991; Loche et al., 1987; Weltman et al., 1994) and exercise induced increases in GH (Eliakim et al., 2006; Vettor et al., 1997). Yet the effects of exercise training on resting GH levels are not consistent, with many studies showing no effects and others showing increases. It is the relationships of GH with obesity and exercise training that will be explored in this section of the chapter.

Obesity

The interaction of GH and adipose tissue is well documented (Rasmussen, 2010; Nam and Marcus, 2000). GH impacts adipose tissue's ability for glucose transport, lipolysis, lipogenesis and preadipocyte (immature adipose cell) differentiation (Nam and Marcus, 2000). The effect of GH on glucose is mediated through IGF-1 in many tissues, however IGF-1 receptors are not present in the mature adipocyte; therefore, the effects of GH on adipose are IGF-1 independent (Shimizu et al., 1986). Evidence suggests that GH acts to inhibit phosphorization of insulin receptor substrate 1 (IRS1) (Smith et al. 1997) and decrease both the number and mRNA of GLUT1 transporters (Kilgour et al., 1995; Smith et al., 1997), limiting insulin's ability for glucose uptake in an adipocyte. More recently, evidence suggests that GH-induced alterations to the insulin signaling transduction at PI3K leads to insulin insensitivity (del Rincon et al., 2007; Barbour et al., 2005). In addition, both in vivo and in vitro treatment of GH suppressed glucose metabolism in the presence and absence of insulin (O'neal et al., 1997; Kilgour et al., 1995; Tai et al., 1990).

The effect of GH on adipocyte precursor cells is equivocal (Hauner et al., 1986; Deslex et al., 1987; Vassaux et al., 1994; Wabitsch et al., 1995). Differences found are most

likely due to different stages of cellular differentiation when GH exposure occurs. In obesity however, GH levels are reduced (Attia et al., 1998; Eliakim et al., 2006; Loche et al., 1987), but elevated levels of GH are required to have an effect on cellular differentiation. In spite of this, children with GH deficiency have reduced fat cell number but increased adipose tissue volume as compared to normal children (Bonnet et al., 1974). Therefore, GH influence on adipocyte differentiation may only influence the number of adipocytes, not total fat mass and is likely not an important influencing factor in the cause or effect of obesity.

Obesity reduces the pulse amplitude of GH in both adults (Iranmanesh et al., 1991; Weltman et al., 1994; Veldhuis et al., 1995) and adolescents (Attia et al., 1998; Eliakim et al., 2006; Loche et al., 1987). Several possible mechanisms exist to explain this decrease in GH secretion including, decreased GH releasing hormone (GHRH) and/or increased somatostatin release, decreased responsiveness of somatotropic cells in the pituitary to GHRH, or a perturbation of the GHRH signal from the hypothalamus to the pituitary (Rasmussen, 2010; Nam and Marcus, 2000). There are two common findings in obesity that could support one or more of these mechanisms. First, increased IGF-1 in obesity could act to enhance the negative feedback on the pituitary and hypothalamus suppressing GH release (Nyomba et al., 1997). Second, elevated levels of circulating free fatty acids (FFA) and/or insulin may negatively influence GH secretion by blocking the release of GH at the pituitary (Casanueva et al, 1987; Clasey et al., 2001). Additionally, insulin increases hepatic sensitivity to GH by increasing the synthesis and translocation of GH receptors to the cell surface (Leung et al., 2000). The increased translocation may lead to a greater IGF1 response in spite of decreased GH. Because IGF1 decreases GH release, the stimulus for GH release is blunted. However, GH returns to normal if weight loss occurs (Rasmussen et al. 1996). Thus, decreased GH in

obesity is an effect, rather than a cause of obesity, as GH pulsatile release returns to normal with weight loss (Rasmussen et al., 1995; Veldhuis et al. 1991).

Fitness

Several studies have shown a positive correlation between GH release and fitness (Eliakim et al., 1996; Vahl et al., 1996; Ubertini et al., 2008; Weltman et al., 1992). Adults with high fitness levels also generally have high insulin sensitivity (Albright et al., 1998; Goodyear and Kahn, 1998; Hawley and Houmard, 2004; Hawley and Lessard, 2008). Increased insulin sensitivity maintains a lower resting insulin level and reduced GH receptor number and affinity; therefore, GH's effect in certain tissues. As Leung et al. (2000) showed with human hepatic GH receptors in vitro, GH binding to its receptor, GH receptor production, and GH receptor presence on the cell surface increases as insulin concentration increases until an insulin concentration of 10 nmol/L. An insulin concentration of 10 nmol/L is well beyond physiological values found in humans, thus it is concluded that insulin likely increases GH sensitivity throughout the physiologic range. The Leung et al. (2000) findings suggest that insulin can increase GH stimulated actions via increased number of GH receptors and GH binding. These findings indicate that insulin increases GH receptor synthesis and translocation to the cell surface in hepatic tissue (Leung et al., 2000). Thus, when insulin levels are reduced, hepatic responsiveness to GH is reduced and increased GH levels are needed to bring about a similar physiologic effect.

There is relatively little data on the relationship between fitness and resting GH in children. However, the two studies that explored this relationship do agree that a positive relationship exists (Eliakim et al., 1996; Kasa-Vubu et al., 2006). Eliakim et al. (1996) used a cross-sectional design to study 23, 15-17 year old females in which components of the GH-

IGF1 axis were correlated with fitness. Results showed a significant positive relationship between maximal oxygen uptake (VO_2max) and 12 hour GH-release (r = 0.36) (samples collected every 20 minutes). Kasa-Vubu and associates, using 37 post pubertal adolescent females (aged 16-21) with a wide range of body fat percentage (17-49%), found that mean 24 hour GH had a significant positive relationship to VO_2max. In addition, this finding was regardless of body composition (as VO_2max increased by one unit, GH increased by three units). Both of these studies used adolescents that had already started puberty, however, a study by Tsolakis et al. (2003) found no difference in GH in prepubescent fit versus unfit boys. These results suggest that the positive relationship may not appear until after puberty begins.

In summary, elevated fitness (VO_2max) can increase the pulse amplitude of GH creating a positive correlation between GH release and fitness (Eliakim et al., 1996; Ubertini et al., 2008 Weltman et al., 1992). However, little is known about the effect fitness has on resting GH, particularly in children.

Exercise Training

One way to improve fitness is through exercise training, although not the focus of this project, training can act as a means to impact resting values of components of the GH-IGF1 axis. Growth hormone does not appear to respond to endurance training in adults. Only a few cross-sectional studies have examined resting GH in trained versus untrained subjects. Bunt et al. (1986) showed no difference between resting GH in seven young adult trained male runners that ran approximately 40 miles per week for at least two years, versus seven untrained males. However, another study comparing seven middle-aged trained men, participating in approximately 10 hrs of cycling a week for at least 10 years, to seven

untrained men, showed that resting GH in trained men was approximately two times higher then the resting GH of untrained men (Manetta et al., 2002). Differences in the findings between these two studies are mostly likely related to the age of the subjects. The Manetta et al. (2002) study used middle aged men (early 50's) whereas the Bunt et al. study (1986) used young men (early 20's). Zaccaria et al.(1999) have shown even trained men in their mid 40's had reduced GH levels compared to young men in their early 20's (~2 pg/mL and ~8 pg/mL, respectfully). Another possible explanation is the small sample size of both studies reducing the generalizability of the findings.

Longitudinal studies may be preferable to cross-sectional studies when exploring the effects of endurance training on resting GH. Several short term training studies evaluating resting GH levels have shown that resting GH is not altered by six (Weltman et al., 1997), seven (Hartley et al., 1972) or ten (Hurley et al., 1990) weeks of endurance training. Zaccaria et al. (1999) looked at both young and middle aged males and found no difference in resting GH in response to 4 months of cycle training. Additionally, a year long training study examining the effect of GH response to training above or below a subjects lactate threshold showed that training above ones lactate threshold can nearly double resting GH release over a 24 hour period (4000 ug/L*min versus 8000 ug/L*min), whereas, training below ones lactate threshold had no effect (Weltman et al., 1992). However, most individuals will likely not train at the intensity required to elicit a change in resting GH. Therefore, based on the results of these endurance training studies GH may not affected by duration of training.

While moderate training does not appear to affect resting GH levels in normal weight adults, there is research to suggest regular exercise can affect levels in obese adults. Irving et

al. (2009) used 34 subjects with metabolic syndrome to determine the effects of a 16 wk low or high-intensity training program versus control. This study showed that regardless of training intensity, GH pulse amplitude increased by ~150%, GH production rate increased ~165%, and GH AUC increased ~150%, whereas controls had no change. Findings indicate endurance exercise training has the capacity to stimulate greater GH production and release in obese individuals. Important to note however, these obese subjects, unlike the normal weight subjects previously discussed, started exercising with GH values lower then normal. Therefore, perhaps exercise allows GH levels to increase to more normal values, but when GH values are already within normal ranges there is little or no effect.

Bunt et al. (1986) showed a possible gender difference in GH response to training. In this study both trained and untrained women were shown to have higher resting GH then men (female controls ~1ng/mL > males; female runners ~1.5 ng/mL > males). When considering mature females and resting GH, phase of menses is an important factor to control for, as the high estrogens during the luteal phase can impact the lipolytic activity, in part by increasing GH release (Ruby et al., 1994). Therefore, the lipolytic nature of estrogen that impacts GH release likely explains differences seen between males and females in the Bunt et al. (1986) study.

Two studies have analyzed the resting GH response to training in children (Eliakim et al., 1996, 1998). Both of these studies employed similar methodology of endurance type activity for ~2.5 hours per session, 5 days a week, for 5 weeks. The only difference was the gender of the subjects. Eliakim et al. (1996) consisted of 20 adolescent females and Eliakim et al. (1998), 23 adolescent males. Neither study found resting GH, GH pulse amplitude, or 12 hour GH release to be altered as a result of 5 weeks of training. However, the relatively

short duration of these studies do not allow for a complete understanding of training induced adaptations. Further, no data on training responses of resting GH in obese adolescents exists.

In summary, exercise training does not appear to affect resting GH in normal weight adults. Gender may affect resting GH, as phase of menses can affect GH release in women (Bunt et al., 1986). In obese adults, exercise training may increase GH, returning it to more normal levels. Finally, there is little to no information on children and the effect of training on resting GH, particularly in obesity.

Insulin-like growth factor 1

Like GH, IGF1 has normal fluctuations influenced by acute activity, diet and circadian rhythm. Since GH is the main stimulus for IGF1, stimuli that increase GH often lead to increases in total IGF1 as well. However, because close to 99% of IGF1 is bound in plasma, changes in the IGFBP can change the physiologic activity of IGF1. For instance, insulin inhibits IGFBP 1 and 2 resulting in increased bioactive or free IGF1 (Holden et al. 1995). Individuals with elevated resting insulin, like many obese individuals, can impact the physiologic activity of IGF1 through minor changes in total IGF1 by influencing free IGF1. Also, those engaging in regular physical activity may have the potential to impact IGF1, as exercise can increase IGF1 (Bang et al., 1990; Kraemer et al., 1990). In this section the relationship between IGF1, obesity, fitness and exercise training will be discussed.

Obesity

IGF1 receptors are not expressed on the mature adipocyte; however this does not mean that IGF1 may not be impacted by obesity. Because the IGF1 stimulating hormone, GH, is reduced in obesity, total IGF1would be expected to decline. Yet, this response has not always been reported. Some research has shown slightly reduced levels (Juul, 2003), no

change (Caufriez et al., 1985; Lukanova et al., 2004), or even slightly elevated levels (Hochberg et al., 1992; Nam et al. 1997) of total IGF1 in obesity.

Free IGF1, not total IGF1, is the physiologically important molecule as free IGF1 binds to the IGF1 receptor. The understanding of how free IGF1 is modified in obesity is unclear. One study has shown free IGF1 to be elevated by ~25% in a group of obese men and women (Frystyk et al., 1999) and two additional studies have shown free IGF1 levels to be elevated by ~33% (Frystyk et al., 1995) and ~40% (Nam et al., 1997) in obese men only. While in several other studies, free IGF1 levels in obese men and women have been shown to be normal (Nyomba et al., 1999; Ricart and Fernadendez-Real, 2001), or low (Gomez et al., 2004). All studies used non-diabetic groups and BMI ranges were similar between the studies that found differing results. Therefore, methodological differences in the measurement of free IGF1 may explain some of the differences found. Two of the three studies that found elevated free IGF1 in obese adults used an in-house, non-competitive monoclonal antibody based-time resolved immuno-fluorometric assay (TR-IFMA) (Frystyk et al., 1995; Frystyk et al., 1999), while the third used a two-site immunoradiometric assay - IRMA (Nam et al., 1997). The IRMA technique was also used in two studies that found no difference in resting free IGF1 in obese versus non-obese subjects. Finally, the only study to find reduced free IGF1 in obese subjects used an enzyme-linked immunosorbent assay - ELISA (Gomez et al., 2004). Rasmussen et al. (2007) however, did attempt to clarify this situation by comparing the ultrafiltrated and dissociated methods of measuring free IGF1, and found both methods to show lower levels in obese women. Thus, it does not seem likely that differences can be explained by the different methodologies used in the separate studies.

Another possible explanation for differences in IGF1 between groups of obese individuals could be differences in fat distribution. Similar to the link between the progression and development of type-2-diabetes mellitus (T2DM) and cardiovascular disease (CVD), increased visceral fat mass has been negatively correlated with total IGF1 (De Pergola et al., 1998; Kunitomi et al., 2002; Rasmussen et al., 1995). An explanation for this apparent relationship could be increased pro-inflammatory mediators, since these have been linked to visceral adipose tissue to obesity, T2DM and CVD (Ritchie and Connell, 2007; Bugianesi et al., 2004).

Differences in free IGF1 may also be linked to disturbances in the IGFBPs that control the amount of free IGF1. Insulin has been shown to inhibit IGFBP1 and 2 (Holden et al., 1995) and may help explain differences in free IGF1 found in obesity. Two studies, however, failed to find a difference in IGFBP3 between normal and obese males (Nam et al., 1997) or females (Rasmussen et al., 2007). More work examining free IGF1 with the IGFBP's is needed to have a better understanding of how and why IGF1 is altered in obesity.

Research examining resting IGF1 in obese adolescents is limited (Eliakim et al., 2006; Kamoda et al. 2005). Kamoda et al. (2005) studied 42 obese adolescents (~12 years) with diabetes mellitus and found free IGF1 to be no different from normal controls; however, IGFBP1 was significantly reduced by ~50% compared to normal controls. Eliakim et al. (2006) studied 25 obese adolescents (~12 years) and found free IGF1 to be non-significantly reduced by 25% compared to normal controls. However, with only 25 subjects the study may not have had sufficient power to detect a difference in free IGF1 that circulates in small quantities. Still IGFBP2 was significantly reduced by 50% but no differences were found in IGFBP3 or 4 compared to normal control. Thus, with limited data, free IGF1 may or may

not be affected by obesity. Thus more data in adolescent populations regarding the relationship between total and free IGF1 is needed to make any conclusions.

Fitness

Limited data exists regarding aerobic fitness and resting total or free IGF1 levels and available information is equivocal. Cross sectional analysis in adults, adjusting for age, show a significant positive relationship between aerobic fitness (VO_2max) and levels of total IGF1 in 42 young men and 26 older men with an r value of 0.64 (Poehlman and Copeland, 1990). Additionally, eight weeks of endurance training in older adults showed resting total IGF1 was increased by 14% with a significant relationship between the change in IGF1 and VO_2max of r = 0.79 (Poehlman et al., 1994). With regard to adolescents, Eliakim et al. (1996), using 23 adolescents, explored this relationship and found a trend in the relationship between fitness and IGF1, r = 0.34, p = 0.054 (Eliakim et al., 1996). One study failed to find any difference between total IGF1 level in 9 highly trained, 28 recreationally trained and 18 sedentary 14-23 year old subjects (Ubertini et al., 2008) indicating a relationship between total IGF1 and fitness may not exist. However, this study had a small n and combined adolescents and young adults, as well as amenorrheic females (38% of all females in the study were amenorrheic). The Ubertini et al. (2008) study also combined athletes of multiple disciplines. The numerous confounding variables, adds considerable hesitation in placing emphasis on their findings. Therefore, associations between aerobic fitness and the total IGF1 may exist in both adults and children but further studies are needed.

Exercise Training

Research on resting IGF1 responses to endurance training in adults has not been consistent. Some studies have reported no effects (Grandys et al., 2008; Vitiello et al., 1997)

while others have reported an elevation (Chicharro et al., 2001; Kokiris et al., 1999; Manetta et al., 2003). Grandys et al. (2008) used healthy young men (early 20's) and had them ride cycle ergometers four times per week at moderate intensities based on their pre-study VO_2max and found no change in total IGF1. Vitiello et al. (1997) used older men and women (mid 60's) and had them run 3-5 times per week, for six months, and also found no change in total IGF1. Both studies used untrained subjects with moderate intensities throughout the training. Research that reported increased total IGF1 used higher intensities and volumes of training for already trained subjects. For example, Chicharro et al. (2001) followed 17 professional riders over a three week race that covered over 3500 km (2170 mi). Subjects were riding at a very high intensity and volume and increased total IGF1 by ~100% across the three week race. Kozoris et al. (1999) followed 14 competitive collegiate swimmers over six months, in which the first four months saw increases in volume of training and finished with two months of progressive tapering. Results of the Kozoris et al. (1999) study showed and increase of total IGF1 by ~30% and free IGF1 by ~700%. Manetta et al. (2003) compared 8 trained cyclists and 8 sedentary controls over four months of intense cycle training and found a 20% increase in total IGF1 after training in the cyclists. Thus, increasing total IGF1 though training may require high intensity, high volume training.

In adults, resting free IGF1 has been shown to be either elevated (Koziris et al., 1999) or reduced (Chicharro et al., 2001) compared to baseline. However, these studies used very different methods. Chicharro et al. (2001), used a 3-week race situation where both volume and intensity was extremely high, and found reductions of free IGF1 of ~32%. The Koziris et al. (1999) study was a six month, controlled training environment, and found increases in free IGF1 throughout the duration of training by as much as ~600%. It is

important to note that the Koziris et al. (1999) subjects had lower free IGF1 levels at the beginning of the study compared to baseline free IGF1 in the Chicharro et al (2001) study (~0.25 and ~0.95 ng/mL, respectfully). In addition, both of these studies had increases in total IGF1; thus, it is interesting to find opposite results with regard to free IGF1. These divergent findings are most likely due the extreme nature of the Chicharro et al. (1999) study compared to the controlled systematic increase in workload with 2 months taper of Koziris et al. (1999). Finding may indicate a link to energy expenditure, metabolic rate, or caloric balance and free IGF1 values. These findings are consistent with Smith et al. (1987) that found total IGF1 declines in response to a negative caloric balance, induced by diet or over exercising.

Findings reviewed to this point led Nemet and Cooper (2002) to suggest an "IGF1 paradox". Nemet and Cooper suggest that early in a training program total IGF1 levels are reduced; this response is similar to a catabolic state. They suggest this is stimulated by increased pro-inflammatory markers. The catabolic state persists until adaptations to the exercise are made, which reduce the production of these pro-inflammatory markers that are inhibiting the GH-IGF1 axis. Therefore, individuals that are moderately trained at the onset of an exercise program have already undergone this adaptation and are able to up-regulate the GH-IGF1 axis immediately, whereas untrained individuals will take longer for increases in IGF1 to occur.

The concept of an "IGF1 paradox" is also supported by data on adolescent males (Eliakim et al., 1998) and females (Eliakim et al., 2001; Eliakim et al., 1996). These studies all used 5 weeks of endurance training in previously untrained adolescents and found reduced levels of total IGF1. According to the "IGF1 paradox", this is caused by increased pro-

inflammatory markers as a result of exercise in untrained subjects. Had these adolescents continued training, muscular adaptations may have occurred allowing for a reduced pro-inflammatory response during exercise. Without the pro-inflammatory response inhibiting GH-IGF1axis, an increase in GH-IGF1 markers can occur.

Insulin

Insulin is a hormone that also can act as growth factor because of its ability to bind to IGF receptors (Denley et al., 2005). Insulin release is stimulated in response to rising concentration of glucose in the blood (Krook et al., 2004; Laron, 2009). Insulin has significant effects on metabolism first of which includes insulin's blood glucose lowering capability through translocation of GLUT receptors to cell surface resulting in cellular uptake of the glucose (Krook et al., 2004; Whiteman et al. 2002). Some of insulin's other effects on metabolism include reducing hormone sensitive lipase (HSL) activity and increasing adipose lipoprotein lipase (LPL) activity (Holt et al., 2003; Jeffcoate, 2002; Lee et al., 1999). These actions reduce the breakdown of adipose triglyceraldehydes (TAG) and increase uptake of circulating TAG by adipose and hepatic tissue for re-esterification (Havel, 2000). Insulin decreases hepatic production of glucose by reducing the rate of glycogenolysis and gluconeogenesis (Holt et al., 2003; Whiteman et al., 2002). Insulin activates enzymes involved in glycogen synthesis and promotes protein synthesis while reducing ketogenesis of amino acids. The interaction of insulin with the GH-IGF1 involves insulin's ability to increase hepatic GH sensitivity (Lueng et al., 2000) leading to a greater IGF1 response which increases protein synthesis.

Obesity

Obese adolescents are widely acknowledged as having a much higher risk for being insulin resistant and developing type 2-diabetes mellitus (T2DM), more so than non-obese adolescents. In fact, Cali and Caprio (2008) have shown that the resting insulin levels of obese children and adolescents are more then twice that of there lean counterparts (~35 μU/mL vs ~10 μU/mL). Among children, research indicates obesity accounts for approximately 55% of the variance in insulin sensitivity (Arslanian and Suprasongsin, 1996). The association between obesity and insulin resistance is believed to be a result of increased circulating free fatty acids (FFA) found in obesity (Girod and Brotman, 2003; Jensen, 2006). FFA circulate in concentrations relative to the amount of adipose tissue; therefore, those with larger fat mass generally have higher levels of FFA circulating at any one time; this greater FFA availability increases gluconeogenesis and glycogenolysis in hepatic tissue (Jensen, 2006). Greater gluconeogenesis and glycogenolysis contribute to an increase in blood glucose. The effect of elevated blood glucose results in a greater stimulus for insulin to be released causing hyperinsulinemia and ensuing insulin resistance (Girod and Brotman, 2003; Jensen, 2006). In normal healthy individuals the actions of insulin on fatty acid metabolism are to 1) stimulate lipoprotein lipase (LPL), which delivers circulating FFA to tissues for re-esterification, 2) inhibit hormone sensitive lipase (HSL), which breaks down stored triacylgyceride, and 3) increase phosphodiesterase which decreases cAMP thus decreasing energy production in the cell and favoring energy storage. However, in obese individuals with insulin resistance the insulin stimulated inhibition of HSL does not occur. The result is constantly active HSL in adipose tissue releasing FFA into the circulation. Increased FFA in

the circulation promotes the storage of FFA as triacyglycerides in non-adipose tissue as well as promotes obesity (Girod and Brotman, 2003).

GH works as an insulin antagonist by inhibiting LPL and stimulating HSL, yet as previous discussed, elevated levels of FFA and insulin can suppress GH release from the pituitary (Casaneuva et al., 1987; Girod and Brotman, 2003). The elevated circulating FFA promotes macrophage infiltration into adipose tissue. Normally adipose is usually populated with only 5% to 10% macrophages; however, diet-induced weight gain can cause infiltration by up to 60% of all cells in adipose tissue (Weisberg et al., 2003). The infiltration by macrophages can increase gene expression and mRNA production of inflammatory cytokines in obese adipose tissue cells. For example, in mice fed a high-fat diet, weight gain is associated with induction of inflammatory pathways. In fact, one study of diet-induced weight gain in mice showed, of the genes upregulated in white adipose tissue, 59% are from inflammation-related genes (Xu et al., 2003). Thus, the elevated insulin seen in obesity is a multifactorial problem which has the potential to affect the GH-IGF1 axis.

Increased levels of insulin seen in obesity may also be linked to increased inflammatory cytokines, as both TNF-α and IL-6 can inhibit insulin's actions (Steinberg et al., 2006; Rotter et al., 2003). The elevated insulin results in an increased number of cell surface GH receptors thus increased sensitivity to GH (Leung et al., 2000). Increased GH sensitivity will reduce the release of GH form the pituitary. Also, because the hyperinsulinemic state potentially increases circulating levels of free IGF1 through inhibition of IGBBP1 and 2, there is a greater inhibition of the stimulus on the pituitary to release GH (Nyomba et al., 1997). Inflammatory cytokines, specifically TNF-α and IL-6, can contribute

to increased insulin; therefore, these cytokines may also have the ability to alter normal function of the GH-IGF1 axis at several places in the axis.

Fitness

Research on adults indicates a strong association between fitness and insulin sensitivity (Lee et al., 2006; Imperatore et al., 2006). This positive relationship is mostly likely because individuals that exhibit high fitness exercise regularly. However, training can result in improved insulin sensitivity independent of improvements in fitness. For example, obese subjects that have impaired insulin function, weight loss may be more beneficial then improving fitness for increasing insulin sensitivity (Katzel et al., 1995). Increased insulin sensitivity, achieved through exercise, allows for reduced free IGF1, therefore increased release of GH at the pituitary (Casanueva et al. 1987). Thus, whether because of increased activity through exercise or increased fitness, insulin sensitivity allows the GH-IGF1 axis to function more normally.

The association between fitness and insulin sensitivity is also evident in children (Kasa-Vubu et al., 2005; McMurray et al., 2000). However, this relationship may have more to do with fat mass than physical activity in adolescents. McMurray et al. (2000) showed that predicted VO_2max in a group of adolescents (182 boys and 167 girls) significantly correlated with resting insulin ($r = 0.654$ in boys and $r = 0.456$ in girls), but did not correlate with leisure time physical activity. Fat mass did have a role, as sum of skinfolds was correlated with insulin ($r = 0.618$ in boys and $r = 0.433$ in girls). McMurray et al. (2000) also demonstrated through 8 weeks of aerobic training, that if VO_2max improved resting insulin was also reduced. These results suggest that if the physical activity stimulus is sufficient to improve VO_2max, it can also affect resting insulin.

Exercise Training

Exercise training can improve insulin sensitivity in untrained adults who are: insulin resistant (Hughes et al., 1993; Rodgers et al., 1988), obese (Gan et al., 2003; Goodpaster et al., 2005), or type 2 diabetic (Bruce et al., 2004; Dela et al., 1995; Kadoglou et al., 2007). However, these adaptations may be the result of regular acute bouts of exercise rather then a combined effect of regular exercise. For example, Dela et al. (1995) studied insulin sensitivity in previously sedentary controls 16 hours after the last training session of a 10-week program of one-leg training. Researchers found that glucose uptake was 30% higher in the trained versus the untrained leg. In addition, the adaption in the trained leg was no longer apparent after 6 days without any training. Similar to these findings, Boule et al. (2005) found 20 weeks of endurance exercise training by 596 untrained men and women significantly improved insulin sensitivity. However, all improvements disappeared within 72 hours of the last exercise bout. The mechanisms involved in the improved insulin sensitivity include both increases in production and activity of several key proteins involved in insulin actions and glucose regulation. The key proteins include, glucose transporter -GLUT4, muscle glycogen synthase, AMPK, IRS1, Akt, and several enzymes of fatty acid oxidation (Hawley and Lessard 2008; Henriksen, 2002; Henriksson, 1995). Exercise training has also been shown to increase insulin sensitivity in adolescents as well (McMurray et al., 2000; Nassis et al., 2005). McMurray et al., (2000) demonstrated that adolescents that had significantly improved their VO_2max following 8 weeks of aerobic training also had reduced resting insulin. Further, Nassis et al. showed in obese adolescents that after 12 weeks of aerobic training insulin levels following an oral glucose tolerance test were significantly reduced at 90 min compared to pre-exercise training. This finding indicates improved insulin

sensitivity. Results indicate that exercise training has the capacity to improve insulin sensitivity in adults and children, particularly if improvements in VO_2max occur.

CYTOKINE IMPACT ON THE GH-IGF1 AXIS IN OBESITY

Tumor Necrosis Factor-α

Tumor necrosis factor-α (TNF-α) is a cytokine involved in systemic inflammation and the stimulation of the acute phase reaction (Abbas et al., 2007). One of the major sources of TNF-α is activated macrophages that have infiltrated adipose tissue (Hotamisligil et al., 1993; Weisberg et al., 2003). TNF-α is synthesized as a membrane protein with both intra and extracellular components. The extracellular component is cleaved by a metalloproteinase releasing a 17 kDa polypeptide into circulation. In circulation, three 17 kDa TNF-α strands polymerize to form one triangular shaped 51 kDa molecule that can bind either type 1 TNF-α receptors or type 2 TNF-α receptors (Abbas et al., 2007). Initiation of the acute inflammatory response to protect from bacteria and other infectious agents is the main role of TNF- α (Beutler et al., 1989), but it is also involved in lipid and glucose metabolism (Grunfeld et al., 1991).

The main physiologic function of TNF-α is to stimulate neutrophil and monocyte adhesion at the site of infection. At relatively low concentrations of TNF-α, the activation of leukocytes at the infection site causes only local inflammation of primarily endothelial cells and is thus, a principal mediator of the acute inflammatory response. If the stimulus for production is sufficient, TNF-α can enter circulation and bind its downstream receptor causing effects in other tissues. In the blood, TNF-α can act on the hypothalamus as an endogenous pyrogen and stimulate fever, on the liver to stimulate an acute-phase response (Abbas et al., 2007), or the pancreas to induce β-cell apoptosis (Eizirik and Mandrup-

Poulsen, 2001). If prolonged production occurs and levels are further elevated, TNF-α can inhibit myocardial contractility, affects vascular smooth muscle function, and inhibits anticoagulant production leading to increased thrombosis. If levels are highly elevated over a long period, TNF-α causes dangerous drops in blood glucose due to over-uptake by the muscle and inability of the liver to restore blood glucose levels (Abbas et al., 2007). Normal healthy individuals with acute low-to-moderate levels of TNF-α, are not likely to have an altered GH-IGF1 axis. However, as levels become chronically elevated interruption of normal lipid and carbohydrate metabolism can occur. By interrupting normal metabolism TNF-α has the potential to impact normal GH function. Furthermore, while increased fitness is associated with lower TNF-α levels (Halle et al., 2004; Ischander et al., 2007), chronically elevated TNF-α levels can impact insulin and thus IGF-1 receptor signaling (Leung et al., 2000). The impact that TNF-α has on obese individuals and those with high fitness levels will be discussed more in depth in the following sections.

Obesity

Childhood and adult obesity is associated with elevated levels of TNF-α (Dedoussis et al., 2010, Halle et al., 2004; Hotamisligil et al., 1993). In fact, TNF-α was originally thought to be released directly from adipose tissue. However, further research has determined that TNF-α is actually released from activated macrophages as a result of increased monocyte infiltration into adipocytes seen in obesity (Weisberg et al., 2003). Elevated levels of TNF-α are one of the links connecting obesity with diabetes (Hotamisligil et al., 1993). Chronically elevated levels of TNF-α have been shown to interrupt intracellular insulin signaling by reducing tyrosine kinase activity. Reducing tyrosine kinase activity affects the insulin receptor and IRS-1 signaling in hepatocytes and adipose tissue, which

leads to insulin insensitivity (Stephens et al., 1997; Ruan et al., 2002; Cai et al., 2005).

Modifying TNF-α or GH has shown to have potential benefits in obesity. One study using obese rodents showed that deletion of TNF-α or its receptors increased insulin sensitivity and glucose tolerance (Uysal et al., 1997). In rheumatoid arthritis patients with elevated resting insulin levels, pharmacologic reduction of TNF-α was shown to reduce insulin from 54.2 μIU/ml to 27.4 μIU/ml (Tam et al., 2007). More directly related to GH, Kubota et al. (2008) found that low dose GH supplementation of rats fed a high fat diet had a 4-fold reduction of TNF-α expression in visceral adipose tissue. These findings suggest that an increase in GH or a reduction in TNF-α has the potential to improve metabolic disturbances common in obesity, such as insulin sensitivity and glucose tolerance.

In obesity TNF-α can impact the GH-IGF1 axis through insulin; direct modification can occur by signaling IRS-1 complex to become phosphorylated on a tyrosine residue preventing its interaction with the IGF1 receptor (Kanety et al., 1995). In mice, TNF-α has been shown to affect insulin signaling by phosphorylating serine (307) (equivalent to serine (312) in human IRS-1) on the IRS-1 complex preventing its interaction with IGF1 or insulin receptor (Gao et al., 2002). Phosporylation of IRS-1 on serine prevents the IGF-1 receptor binding and targets the IRS-1 for degradation (Pederson et al., 2001) therefore, stopping the propagation of the IGF-1 or insulin signal. The effects of TNF-α on the interruption of IGF-1 and insulin are believed to involve inhibitor of κβ kinase (Gao et al., 2002) and c-Jun NH2-terminal kinase which are both downstream of the TNF-α receptor (Strle et al., 2006). Therefore TNF-α has the potential to induce a state of IGF-1 resistance in affected cells, which may also account for some of the elevated values of free IGF1 seen in obese adult subjects.

Fitness and Exercise Training

An inverse relationship between fitness and general inflammation has been well established in adults (Beavers et al., 2010; Hamer et al., 2007; Thomas and Williams, 2008). However, a clear establishment of an inverse relationship between TNF-α and fitness is lacking. Arsenault et al., (2009) did not find a relationship between TNF-α and fitness (VO_2max) in adults. The lack of a significant relationship in the Arsenault et al. (2009) study may have to do with what portion of the fat mass was included in the analysis. Researchers only included visceral adipose tissue in their study. TNF-α, however, has been shown to be equally produced in both visceral and subcutaneous adipose tissue (Winkler et al., 2003). Ignoring the subcutaneous depot of adipose could have altered the relationship between circulating TNF-α and total body fat. Therefore, the lack of relationship between TNF-α and fitness is believed to occur for two reasons, 1) the relationship has not been extensively researched and 2) when it has been explored, a confounding variable may have prevented a relationship from being determined. The current project will both add more information about the relationship between TNF-α and fitness as well as attempt to control for potential problems in the design of previous studies design by not partitioning adipose stores that both contribute to circulating levels of TNF-α.

A significant relationship between fitness and TNF-α has been found in adolescents (Halle et al., 2004; Ischander et al., 2007). Halle et al. (2004) divided 197 children and adolescents into 4 groups similar to the presently proposed study (obese fit, obese unfit, normal fit, and normal unfit) according to BMI and max METs. They found that obese fit subjects had similar TNF-α levels compared to normal fit, whereas the obese unfit subjects had similar TNF-α level compared to the normal unfit group. Halle et al. (2004) concluded

that TNF-α levels are primarily determined by fitness level. Additionally, Ischander et al. (2007) matched for age and BMI percentile in two groups (elevated fitness and sedentary) of adolescent females and showed that the group with elevated physical fitness had resting TNF-α values significantly less (~25%) than that of the sedentary group. This study shows the potential for elevated TNF-α in adolescent females with reduced levels of fitness independent of weight status. Together these studies suggest obese individuals may be able to reduce levels of TNF-α by improving their fitness.

Long term training studies in sedentary adults with elevated TNF-α levels at baseline are not all in agreement regarding the response to the training. Kohut et al. (2006) used older subjects (70's) who were overweight, not obese, therefore only had TNF-α values slightly above normal (~2.5 pg/mL). Researchers still saw a significant drop in TNF-α (~1 pg/mL) and a significant increase in max METS (measure of cardiorespiratory fitness) in the 10 month endurance training protocol. Kondo et al. (2006) used young (18-23) obese adults with basal TNF-α values above 7.5 pg/mL. Seven months of endurance training significantly improved fitness (VO_2max) and reduced TNF-α levels to ~ 4.5pg/mL. Although there was a significant drop in TNF-α, it was still elevated above normal. Conversely some studies found no changes in TNF-α, but those studies may have been either too short in duration (6-12 weeks) to significantly reduce body fat (Stewart et al., 2005 and 2007) or did not report any fitness data in order to evaluate the effectiveness of the training program (Nicklas et al., 2004). The study by Nicklas et al. (2004) was 18 months in duration and saw weight loss through diet alone as well as through diet and exercise, but still saw no changes in TNF-α. Therefore, improved fitness may be a prerequisite for reduced TNF-α levels, when levels are elevated above normal. However, Kadogluo et al. (2007) had subjects train for 6 months and

had significant increases in fitness but TNF-α was only reduced by a non-significant margin (~ 1pg/mL). Based on these results it appears that in order to reduce TNF-α in adults, they must start with elevated levels and training causes both a significant drop in weight and improvement in fitness.

Interluekin-6

Interluekin-6 (IL-6) is a cytokine that can have pro-inflammatory and anti-inflammatory effects (Abbas et al., 2007). IL-6 is produced primarily by activated macrophages, vascular endothelial cells, fibroblasts, and muscle (Febbraio and Pederson, 2005). IL-6 can also be produced by other cells in response to microbes and cytokines such and IL-1and TNF-α (Mendall et al., 1997). The circulating form of IL-6 is a homodimer of 185 amino acids bound to a cytokine-binding protein called gp130. The IL-6 signaling pathway is known to involve activation of JAK and STAT (Abbas et al., 2007) which are also involved in the GH and IGF1 intracellular activation pathways (Kopchick et al., 1999).

Interleukin-6 is believed to have both pro- and anti-inflammatory effects, dependent upon the stimulus and site of release. Pro-inflammatory mechanisms for IL-6 are linked to its involvement in innate and adaptive immunity. These mechanisms include increasing the production of acute phase proteins by hepatocytes and neutophils from bone marrow in innate immunity (Abbas et al., 2007). In adaptive immunity, pro-inflammatory IL-6 can stimulate pro-inflammatory cytokines, such as IL-17, by inhibiting regulatory T-cells. Anti-inflammatory effects of IL-6 are achieved through a reduction in TNF-α secretion during non-damaging exercise (Schindler et al., 1990; Starkie et al., 2003) and stimulation of the anti-inflammatory cytokines IL-1ra and IL-10 (Febbraio and Pederson, 2005; Steensburg et al., 2003). Mechanisms involved in anti-inflammatory muscle IL-6 production are believed

to involve reductions in intramuscular glycogen content. As glycogen levels are reduced IL-6 production increases ultimately resulting in increased AMPK activation, which increases glucose uptake and fatty acid oxidation, thus a sparing effect on glycogen (Febbraio and Pederson, 2005). In the following, the impact that IL-6 has on obesity and those with high fitness levels will be discussed more in depth.

Obesity

Obesity has been shown to be directly related to circulating levels of IL-6 with adipose tissue being a major site of IL-6 production (Fried et al., 1998; Shoelson et al., 2006). Cells of the adipose tissue matrix, stromal vascular cells and adipocytes themselves can produce IL-6 (Eder et al., 2009; Fried et al., 1998). In addition, the release of IL-6 by adipose tissue, like that of TNF-α, originates from activated macrophages that have infiltrated adipose tissue in response to elevated adiposity (Eder et al., 2009). The activated macrophage release of IL-6 contributes significantly to the adipose tissue derived IL-6 (Weisberg et al., 2003). The association of inflammatory IL-6 and adiposity is believe to be reduced oxygen availability in areas of fat depots as tissue mass increases during the progression of obesity (Eder et al., 2009). When levels of IL-6 are chronically elevated, as seen in obesity, IL-6 can promote its own production in vitro and inhibit anti-inflammatory cytokines (Fasshauer et al., 2003). Anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist elicit much of their effect through stimulation of additional anti-inflammatory hormones. Therefore, a reduction in these anti-inflammatory cytokines results in a reduction of the anti-inflammatory hormone adiponectin, resulting in obesity being a chronic low grade inflammatory state (Fantuzzi, 2005).

The source and length of IL-6 exposure may be linked to function and effect. Acute release of muscle-derived, exercise-induced IL-6 has been linked to regulation of glucose metabolism and release during exercise (Pederson, 2007). Chronic release of IL-6 from adipose tissue in response to decreased insulin sensitivity or inactivity can have several potential effects on glucose utilization, such as inhibited adiponectin release and activation of suppressors of cytokine signaling (Hajri et al., 2010; Tan et al, 2005). Suppressors of cytokine signaling or SOCS are a family of proteins that regulate the strength and duration of the cytokine signaling cascade. The SOCS family of proteins also link IL-6 activation with the GH-IGF1 axis. One mechanism that may be involved in the IL-6 - IGF-1 signaling interaction is the shared intracellular signaling molecule JAK/STAT (Takahashi et al., 1999). In the IL-6 pathway, STAT will increase the expression of multiple suppressors of cytokine signaling - SOCS (Tan et al., 2005). In particular, SOCS-3 along with direct action of IL-6, can interrupt both GH and IGF-1 intracellular signaling (Tan et al., 2005; Rui et al., 2002). Thus, the SOCS produced via IL-6 is believed to be involved in the interruption of the anabolic pathways of IGF-1 and GH (Haddad et al., 2005).

Several studies have shown IL-6 to be elevated in obese children and adolescents (Halle et al., 2004; Russell et al., 2009). Halle et al. (2004) split 197 children and adolescents (age 10-14) into obese and non-obese groups based on BMI and found the obese group had elevated IL-6. Russell et al. (2009) matched 15 obese subjects with normal weight adolescents and found significantly elevated IL-6 in the obese group, which was negatively associated with GH release. In both papers the authors concluded that the obese state of the adolescents were primarily responsible for the elevated level of inflammation. Russell et al.

(2009) extended their findings and concluded that elevations of inflammatory markers, including IL-6, can be predicted by a state of GH reduction or deficiency.

Fitness and exercise training

Much like increasing one's fitness can reduce levels of IL-6, those with elevated levels of fitness have been associated with lower levels of resting IL-6. In cross-sectional analysis, Kullo et al. (2007) studied 173 adult men (26-84 yrs) with low risk for coronary heart disease and found that VO_2max was negatively associated with IL-6 ($r=-0.38$; $p = 0.0001$). This study indicates a role for fitness in the prevention of obesity related inflammation.

Several studies have shown the relationship between fitness and resting IL-6 in children and adolescents (Balagopal et al., 2005; Halle et al., 2004; Ischander et al., 2007). Halle et al. divided 197 children and adolescents into 4 groups similar to the presently proposed study (obese fit, obese unfit, normal fit, and normal unfit) according to BMI and max METs. They found that obese fit subjects had similar IL-6 levels compared to normal fit and normal unfit subjects, but obese unfit subjects had elevated IL-6 compared to the other three groups. Halle et al. (2004) concluded that the elevated IL-6 of the obese unfit group only indicates that improved fitness can counteract the some of the effects of obesity. Ischander et al. (2007) matched 37 active 14-17 year old girls with BMI matched sedentary girls to compare fitness, fat mass and inflammatory markers, including IL-6. As expected researchers found the active girls had significantly elevated fitness level and reduced fat mass and resting IL-6 levels compared to the sedentary girls. Ischander et al. (2007) concluded the reduced fat mass in the active group was due to the increased amount of regular physical activity and elevated IL-6 levels in the sedentary girls was due to elevated fat mass, even

though BMI between groups was equal. Additionally, longitudinal analysis in adolescents has shown IL-6 to be reduced with weight reduction (Balagopal et al., 2005). These researchers had 8 obese adolescents do 45 min of moderate intensity exercise three times per week and gave subjects nutritional education and guidance throughout the study. Collectively, these studies suggest resting IL-6 in adolescents may be predicted by fitness but also can be modified through diet and exercise.

Numerous randomized controlled trials have found that exercise training reduces resting IL-6 in various populations (Stewart et al., 2005; Kohut et al., 2006; Balagopal et al., 2005; Esposito et al., 2003; Nicklas et al., 2004; Balducci et al., 2009). Stewart et al. (2005) exercised 17 inactive overweight adult subjects for 12 weeks using a combined endurance and resistance training program that resulted in significant increases in fitness and weight loss and reduction in stimulated IL-6 release by ~30%. Similar results were found by a two year diet and exercise program of 60 obese women by Esposito et al. (2003). They reported weight loss with a concomitant reduction of IL-6 from 4.3 pg/mL to 2.9 pg/mL. Two studies however, were able to achieve a significant reduction in IL-6 and improvement in fitness independent of any significant reduction in weight (Balducci et al., 2009; Kohut et al., 2006). Balducci et al., (2009) study used 20 sedentary diabetic patient's and used endurance training at moderate intensities for 60 min for 12 months. Kohut et al. (2006) used 19 overweight subjects, and they did not significantly reduce weight over 10 months of endurance training. Both studies found significant reductions in IL-6. A direct effect of improved fitness reducing IL-6 can be made, and in these studies it appears to be more important then reducing weight. Therefore, although weight loss may be of benefit, it is not required to have a favorable effect on resting IL-6 levels.

The effects that exercise training has to reduce IL-6 in obesity, can affect both GH and IGF1. Denson et al., (2003) used IL-6 null mice compared to wild type mice to show the affect on GH. In this research Denson et al. (2003) showed lipopolysaccharide pretreatment prevented GH signaling in hepatic tissue in wild type mice; however, GH signaling was preserved in IL-6 null mice, indicating IL-6 involvement in the inhibition of GH signaling. Additionally, De Benedetti et al. (1997) used transgenic mice treated since the early phases of life with a specific NSE/hIL-6 strain that overexpresses IL-6. This construct was used to demonstrate that IL-6 overexpression reduced IGF1 compared to wild type mice. Also, when De Benedetti's group treated the wild type mice with the same NSE/hIL-6 strain that was used on the transgenic IL-6 overexpression mice, IGF1 was reduced. The Denson et al. (2003) and De Benedetti et al. (1997) studies demonstrate that IL-6 has the capacity to reduce GH and IGF1. Thus, individuals that use exercise training, increased fitness, or increased physical activity to reduce IL-6 levels can mitigate potential alterations to the GH-IGF1 axis.

SUMMARY

This review has highlighted several key factors in the GH-IGF1 axis that are important to our understanding of the effects that obesity can have on the axis. GH has been shown to be reduced in obesity in both children and adults. Free IGF1 is generally elevated in obesity, due in part to elevated levels of resting insulin seen in both obese adults and obese children. However, the individual response of total and free IGF1 in obese children is currently not clear. The elevated levels of resting insulin in obesity are mainly a function of the elevated levels of adipose tissue and FFAs which can interrupt insulin receptor binding and intracellular signaling. Further research is needed, particularly in children, to understand the effect of obesity on the GH-IGF1 axis and insulin as these alterations may play a role in

the propagation of obesity. Additionally, by using the groups proposed in this study, differences in fitness and obesity status will be able to be determined.

Little work has been done looking at the GH-IGF1 axis in childhood obesity as it relates to inflammation. Inflammation has been demonstrated as a potential mechanism for alteration of the GH-IGF1 axis. IL-6 and TNF-α were demonstrated as two inflammatory cytokines that can have effects on the GH-IGF1 axis through their interruption of insulin signaling which can result in a decrease in GH and an increase in free IGF1. Additionally, IL-6 can interrupt the axis by disrupting the IGF1 intracellular pathway. No previous work has also accounted for the level of fitness of the subjects as a potential influence upon this relationship, let alone using the unique method of removing fat mass when calculating fitness. This unique approach allows for comparison of obese and non-obese subjects based on fitness, something previously very difficult. However, the more common unit (mL/kg/min) will be used as well to allow for comparison of this research to other findings.

The proposed project will be able to answer several important questions that will provide further information and understanding of the interaction between fitness level, obesity status, the GH-IGF1 axis, and obesity related inflammation. With this information research can begin to explore the effects and potential positive outcomes of improved fitness in obese populations especially in children.

CHAPTER THREE

MANUSCRIPT ONE

Differences in the GH-IGF1 axis in children of different weight and fitness status

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ABSTRACT

Background: Obesity continues to be a major problem for youth and can lead to a disruption of the growth hormone – insulin-like growth factor 1 axis (GH-IGF1). Higher aerobic fitness may be associated witha more normal state of the GH-IGF1 axis, but this relationship has not been explored when controlling for differences in weight and fitness status.

Objective: To determine if differences in the GH-IGF1 axis exist between youth of high and low fitness who are obese or of normal weight.

Methods: 124 children (ages 8-11) divided into four groups based on BMI and VO_2max (mL fat free mass/ min): normal and high fit (NH), normal and low fit (NL), obese and high fit (OH), and obese and low fit (OL). All had height, weight, skinfolds, body mass index (BMI), body fat percentage and predicted VO_2max assessed. Resting growth hormone (GH), total insulin-like growth factor 1 (total IGF1), free insulin-like growth factor 1(free IGF1), and insulin were obtained from a fasting blood sample.

Results: GH was significantly greater in the NH group compared to the NL group only. Total IGF1 or free IGF1 were not different between any of the groups. Insulin was greater in the OH and OL groups compared to the NH and NL groups. However, only insulin and free IGF1 were significantly related to fitness (ml/kg/min and ml/kg_{FFM}/min).

Conclusions: Fitness may have the potential to mitigate some of the obesity related reduction of GH that may be involved with continued weight gain.

Keywords:

Insulin, fat free mass, VO_2max,

INTRODUCTION

Obesity leads to a disruption of the GH-IGF1 axis (Bray and Bouchard, 2004). Part of the disruption can be linked to the increased insulin that often accompanies obesity. Insulin increases the number of cell surface GH receptors, increasing hepatic tissue sensitivity to GH (Leung et al., 2000). The result of increased hepatic sensitivity is a decreased amount of GH needed to stimulate IGF1 release. Elevated levels of insulin can also affect levels of free IGF1 by inhibiting some of the binding proteins for IGF1 (Frystyk et al., 1999, Nam et al., 1997, Nyomba et al., 1997). These mechanisms result in obese children having low levels of resting GH with normal being 2-5 ng/mL and normal levels of total IGF1and free IGF1 being between 200-400 ng/mL and 2-4 ng/mL, respectfully (Kamoda et al., 2006; Eliakim et al., 2006).

In the USA, the most recent estimates show that 18.7% of all children age 6-19 are obese (defined as $\geq 95^{th}$ percentile) (Ogden, 2010). Additionally, the percent of boys in the the most extreme BMI group continues to increase (Ogden, 2010). Hormonal alterations of the GH-IGF1 axis in obesity may play a role in exacerbating the problem in overweight individuals because of the axis's involvement in fat utilization and muscle development (Alexopoulou et al., 2010; Vijayakumar et al, 2010). Therefore, research exploring factors that potentially contribute to the problem of continued weight gain is needed. The purpose of this investigation was to determine if differences in the GH-IGF1 axis of children exist between obese and normal weight adolescents with high and low fitness

METHODS

Subjects

Subjects were obtained from the Cardiovascular Health in Children III (CHIC III) study, Cohort 5 (J.S. Harrell, P.I.). The CHIC III study investigated metabolic syndrome and cardiovascular risk factors in youth from rural North Carolina. A total of 124 children and adolescents were selected from the 1486 participants, based on their weight and fitness status. Of the original 1486 participants, the mean age was 9.7 ± 1.1 years old. The sex and race distribution was ~50% male, 50% female, 55% African-American, 37% Caucasian, and 8% other races. Prior to participation parents and child gave written consent and assent in accordance with the IRB of the University of North Carolina at Chapel Hill.

The present investigation included subjects aged 10.0 ± 0.9, 41.9% male, 58.1% female, 58.1% African American, 31.5% white, and 10.4% other races. Subjects were divided into 4 groups based upon their weight and aerobic fitness (VO_2max) status: normal weight high-fit (NH), normal weight low-fit (NL), obese high-fit (OH), and obese low-fit (OL). Normal weight was defined as $<85^{th}$ and $>5^{th}$ BMI percentile for age and sex. Obese was defined as $\geq 95^{th}$ BMI percentile for age and sex. Subjects in the obese groups were matched according to sex and pubertal status with normal weight subjects while only including subjects in Tanner stage 1-3.

Aerobic fitness was determined based upon estimated fat free VO_2max express per unit of fat free mass ($mL/kg_{FFM}/min$). This measurement was used in place of the more common mg/kg/min to account for the differing levels of adiposity between the groups. Using VO_2max expressed in units $mL/kg_{FFM}/min$ allows for comparison of oxygen uptake excluding body fat and is based more on metabolically active tissue; therefore, allowing for

better comparisons of fitness between the subjects of different weight status. Aerobic fitness levels were developed (unpublished data; Hosick) based on data from 3235 CHIC subjects aged 8-12 collected from 1992-2005. From that data the 33rd and 66th percentiles were determined by age and gender. The results are shown for males (Table 1) and females (Table 2). Subjects that had a VO_2max (mL/kg_{FFM}/min) less than the 33rd percentile were included in the low-fitness group; those with a VO_2max (mL/kg_{FFM}/min) greater than the 66th percentile were included in the high fitness group.

Table 1. The 33rd% (low-fit) and 66th% (high-fit) cut-points of the boys presented by age.

n	Age	Fitness 33rd% (mL/kg_{FFM}/min)	Fitness 66th% (mL/kg_{FFM}/min)
357	8	51.8	59.3
467	9	52.4	59.8
413	10	49.2	57.8
205	11	47.8	55.7
192	12	47.1	53.2

Table 2. The 33rd% (low-fit) and 66th% (high-fit) cut-points of the girls presented by age.

n	Age	Fitness 33rd% (mL/kg_{FFM}/min)	Fitness 66th% (mL/kg_{FFM}/min)
420	8	49.9	58.7
453	9	49.8	57.1
357	10	46.0	53.8
188	11	43.4	50.4
183	12	45.5	52.0

Data collection procedures

Complete details of the data collection procedures are presented elsewhere (McMurray et al., 2000). In summary, all data was collected in the subject's school during the school day with the exception of blood draws, which were collected in the morning. Height was measured using a standard calibrated stadiometer (Perspective Enterprises,

Portage, MI) and body mass measured using a calibrated electronic scale (Model 2101KL, Healthometer Medical, Bridgewater. IL). Body mass index (BMI) was calculated using the standard formula: mass (kg)/height $(m)^2$. Skinfolds were measured at the triceps and subscapula in triplicate (NHANES III, 1974) using calibrated Lange Skinfold calipers (Cambridge Scientific, Cambridge, MD), and used to estimate body fat percentages using sex and age specific formulas (Slaughter et al., 1998). Pubertal status was estimated using the Pubertal Development Scale, a sex-specific, self-administered questionnaire with 5-item subscales (Petersen et al., 1988)

Aerobic fitness (VO_2max) was estimated using previously determined methods of McMurray et al. (1998). Heart rate was measured using a Polar Pacer heart rate monitor that was calibrated against an electrocardiogram. Cycle ergometers used were a BodyGuard (model 990), Tunturi magnetic-braked Tunuri Oy Ltd., (Turku, Finland), or a Monarch (model 818; Monark, Varberg, Sweden). Subjects pedaled the ergometer at a rate of 60 rpm for three, 3-min stages. The workload during subsequent stages was increased by 30 to 60 W, depending on the subjects age or heart rate at the end of the first stage. Heart rates were measured the last 10 seconds of each minute throughout the test. Heart rates measured during the last minute of each stage were used to extrapolate a physical work capacity (PWC). The PWC was converted to oxygen uptake (L/min) using equations established by McMurray et al. (1998). This method has produced an $r = 0.80$ with measured VO_2max.

The VO_2max per kilogram of the fat free mass (VO_2FFM) was determined from estimations of body fat percentage and absolute VO_2max. Fat free mass (FFM) was determined by subtracting predicted body fat percentage from one and multiplying by the subjects total body mass (kg). Finally, the subjects FFM was divided by absolute VO_2max

giving the unit mL of O_2 per kilogram of fat free mass per min (VO_2FFM). For comparison with other studies, VO_2max was also predicted in units of millimeters of oxygen per kilogram total body mass (VO_2kg).

Blood analysis

Subjects were called the day before and reminded not to eat anything and drink only water until after the blood draw. Upon arrival the next morning subjects confirmed they fasted. All blood samples were obtained using the antecubital space of the arm of the subject's preference. Samples were immediately centrifuged; plasma or serum separated into individual mircocentrifuge tubes with ~0.5 ml plasma per tube. Samples were then placed on dry ice and transported to the Applied Physiology Laboratory on the campus of the University of North Carolina, where they were stored at -80˚C until analysis.

All blood analysis used commercially available assay kits. Serum total GH values were determined using ELISA technique (IBL-America, Minneapolis, MN). The intra-assay coefficient of variation (CV) for GH was 6.1%; with an inter-assay CV of 4.0%. The sensitivity reported from IBL-America was 0.2 ng/mL. Total IGF1 (total IGF1) values were measured using ELISA technique (R&D System Laboratories (Minneapolis, MN). The sensitivity of the total IGF1 assay was reported as 0.026 ng/mL. The intra-assay CV for total IGF1 was 3.7%; with an inter-assay CV of 4.1%. Free IGF1 (free IGF1) concentration was determined in EDTA plasma using ELISA technique (Diagnostics Systems Laboratories, Webster, TX) and had a sensitivity 0.015 ng/mL. The intra-assay CV for free IGF1 was 4.3%; while the inter-assay CV was reported as 10.2%. Plasma insulin levels were determined in duplicate using a commercially available kit (Linco, St. Charles, Mo., USA). Linco reports a 0.02% cross-reactivity with proinsulin, glucagon, somatostatin, IGF-1 or

pancreatic polypeptide. Our CV between duplicate samples was less than 8% for measured insulin values.

Statistical analysis

Means and SEM were computed for all variables by group (NH, NL, OH, OL). To determine if differences existed by sex and race in the sample analysis of variance (ANOVA) was conducted for all hormones. For variables that produced significant differences, an analysis of co-variance (ANCOVA) was used to determine group difference controlling for sex and race. For variables that did not differ by sex and or race an ANCOVA was used to determine differences by group. When the ANCOVA or ANOVA analyses showed significant differences between groups a Tukey post-hoc test was applied to compare specific means. To further explore any inter-relationships partial Spearman correlations were run between the hormonal concentrations and measures of fitness (VO_2FFM and VO_2kg). Spearman correlations were run because normality of our measure cannot be assumed due to the polarilty involved in the group selection. Partial correlations adjusting for differences body fat percentage were run. Body fat percentage was adjusted for by adding it to the model first to account of any influence that body fat may have on these relationships. The alpha level was set at $p<0.05$. All statistical analysis was computed using SAS version 9.1 (Cary, NC).

RESULTS

Group characteristics

The group characteristics are found in Table 3. The groups did not differ significantly by age, sex, race or pubertal status. Both obese groups had significantly greater

($p<0.05$) height, mass, BMI, BMI percentile, fat percentage and fat free mass, compared to the NH group. The obese groups, regardless of fitness status were taller than the normal weight groups, in particular, the NH group ($p < 0.05$). Both high-fit groups had significantly elevated VO_2FFM compared to the low fitness groups. The VO_2kg was significantly different between all groups in the following order from highest to lowest: NH, NL, OH, OL.

Group comparisons of hormones

The results of the ANOVA test to determine if hormones were different across gender and race showed that total and free IGF1 were elevated in African Americans compared to whites (total IGF1: $p=0.03$; free IGF1: $p=0.0003$) and in boys compared to girls (total IGF1: $p<0.0001$; free IGF1: $p<0.0001$); thus, a ANCOVA adjusting for sex and race was used for total and free IGF1 whereas ANOVA was used for GH and insulin.

Growth hormone was significantly elevated in the NH group compared to the OL group (Figure 1). No differences were found between any other groups. Total IGF1, after controlling for differences in gender and race, was not significantly different between any of the groups ($p=0.530$; Figure 2). Free IGF1 was not significantly different between any of the groups after controlling for gender and race ($p=0.189$; Figure 3). Insulin was significantly lower in the NH and NL group than in the OH and OL groups (Figure 4).

Table 3. Mean ± standard deviation of anthropometric and fitness variables presented by group.

	Normal High Fitness	Normal Low Fitness	Obese High Fitness	Obese Low Fitness
N	31	31	31	31
Sex (female, male)	13, 18	13, 18	13, 18	13, 18
Race (AA, W, O)	19, 8, 4	20, 7, 4	13, 16, 2	20, 8, 3
Age (yrs)	9.9±0.9	10.1±1.0	10.1±0.8	10.0±0.9
Tanner Stage	2.2±0.7	2.3±0.7	2.3±0.7	2.3±0.7
Height (cm)	139±8.1†‡	143±9.3	147±9.4*	148±9.1*
Body Mass (kg)	33.9±5.2†‡	36.7±6.5†‡	60.9±17.4*#	61.2±11.2*#
BMI (kg/m^2)	17.4±5.7†‡	17.6±1.7†‡	27.7±4.9*#	27.7±3.4*#
BMI percentile	53.2±20.8†‡	56.8±24.0†‡	97.8±1.5*#	98.1±1.2*#
Body Fat percentage	17.4±5.7†‡	16.0±6.6†‡	36.7±11.0*#	32.2±7.3*#
Fat Free Mass (kg)	27.9±4.1†‡	30.8±5.4†‡	37.1±7.4*#	40.9±6.0*#
VO_2FFM ($mL/kg_{FFM}/min$)	62.1±8.1‡#	35.4±6.6†*	64.8±10.3‡#	35.1±6.6†*
VO_2kg (mL/kg/min)	51.3±7.5#†‡	29.8±6.4*†‡	40.8±9.0*#‡	23.9±5.6*#†

AA = African American, W = White, O = Other races. * $p<0.05$ from NH, # $p<0.05$ from NL, † $p<0.05$ from OH, ‡ $p<0.05$ from OL

Figure 1. Mean (±SEM) resting growth hormone level of the normal weight high-fit (NH), normal weight low-fit (NL), obese high-fit (OH), and obese low-fit (OL) groups.

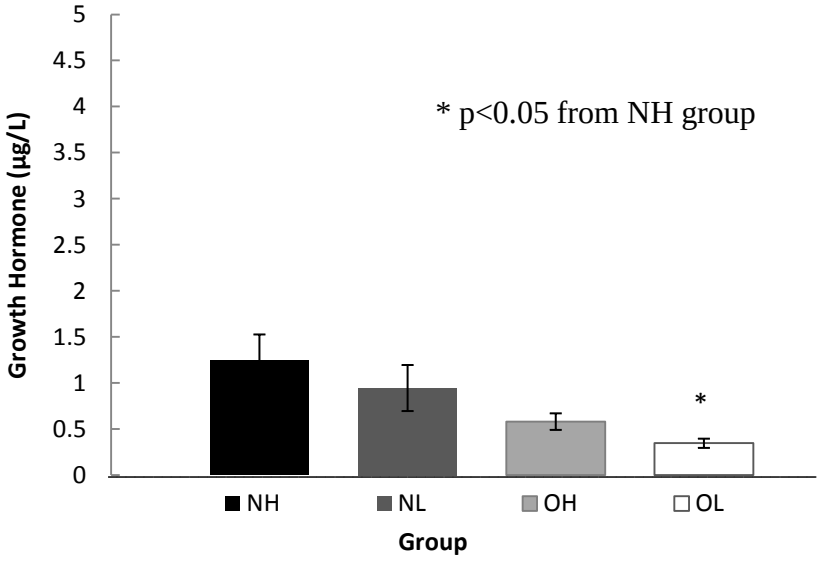


Figure 2. Mean (±SEM) resting total IGF1 level of the normal weight high-fit (NH), normal weight low-fit (NL), obese high-fit (OH), and obese low-fit (OL) groups.

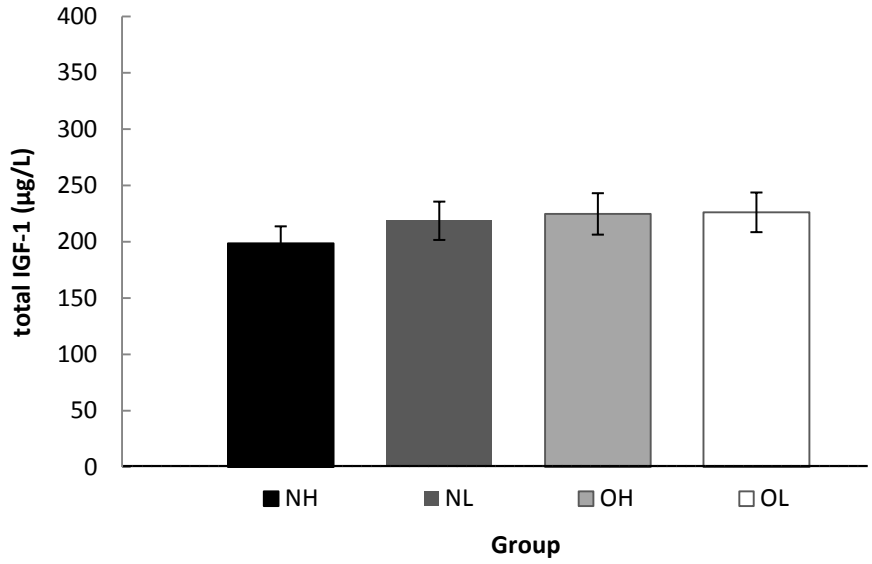


Figure 3. Mean (±SEM) resting free IGF1 level of the normal weight high-fit (NH), normal weight low-fit (NL), obese high-fit (OH), and obese low-fit (OL) groups.

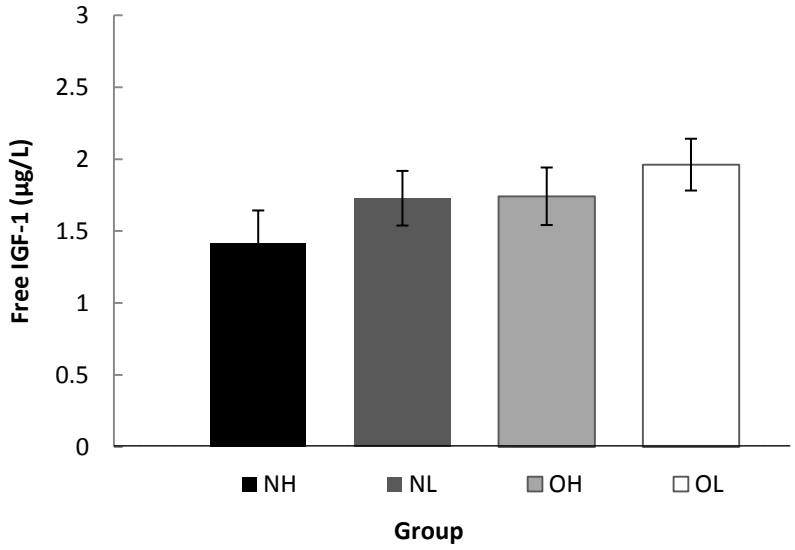
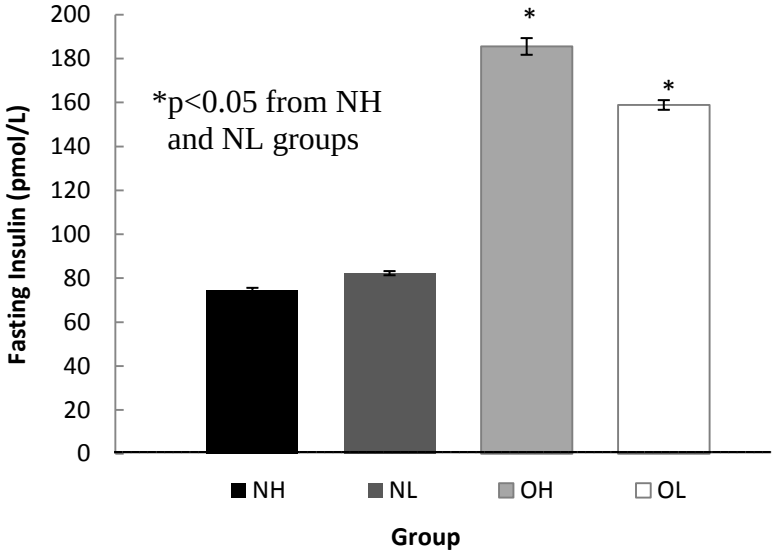


Figure 4. Mean (±SEM) fasting insulin level of the normal weight high-fit (NH), normal weight low-fit (NL), obese high-fit (OH), and obese low-fit (OL) groups.



Relationships between hormones and fitness variables

Partial spearman correlations adjusting for difference in body fat percentage are reported in Table 4. In brief, insulin was significantly correlated to free IGF1, VO2FFM and VO2kg. Both total IGF1 and free IGF1 had a significant relationship with GH, but only free IGF1 was related to the aerobic fitness measures.

Table 4. Partial spearman correlations between insulin, growth hormone, total IGF1, free IGF1and fitness measures, adjusting for body fat

	GH	total IGF1	free IGF1	VO_2FFM	VO_2kg
Insulin	0.053	0.145	**0.199***	**-0.212***	**-0.226***
GH		**0.221***	**0.204***	0.145	0.147
total IGF1			**0.568**$^{\#}$	-0.159	-0.107
free IGF1				**-0.272***	**-0.219**$^{\#}$

*p<0.05, $^{\#}$p<0.01

DISCUSSION

Alterations to GH-IGF1 axis in obesity are evident (Bray and Bouchard, 2004). These alterations have mechanistic links to alterations in insulin sensitivity also seen in obesity (Frystyk et al., 1999; Nam et al., 1997; Nyomba et al., 1997). Studies that have shown improved fitness in obese individuals have also shown improvements to insulin sensitivity that may be favorable for functioning of the GH-IGF1 axis (Bell et al., 2007; Chang et al., 2008; Landt et al., 1985; Nassis et al., 2005). This study was designed to examine the relationship between fitness, obesity and the GH-IGF1 axis. Our results suggest that having a higher VO_2max in obesity does not protect against insulin resistance but can diminish some of the obesity related reduction of GH.

Adolescents in the OL group had significantly lower GH compared to the NH groups, whereas subjects in the OH group were not different from any group. However, further assessment of Figure 1 shows mean GH level of the NL and OH groups each were non-significantly lower compared to the NH group. Thus, the high fitness level of the OH group may mitigate the lowering GH that occurred had the OH group not been highly fit. Together these results suggest the effect of obesity is greater than the effect of fitness on alterations to GH. The lower GH in the OL group was expected due to the elevated levels of insulin also present, which can increase the sensitivity of hepatic tissue to GH (Leung et al. 2000). If the relationship between fitness and GH was driven by insulin alone, then the OH group should have significantly lower levels of GH as well, given that the OH and OL groups had similar resting insulin. Because the OL and OH groups were similar with the exception of fitness level the non-significant reduction of GH in the OH group may have been due to their greater fitness level; yet, a significant association between GH and fitness was not found. Perhaps

the combination of fitness and insulin level diminish the reduction of GH as fitness has a significant negative correlation with insulin. However, a posteriori analysis correlating GH with VO_2FFM and insulin did not show a significant relationship (p=0.38). Thus, we are unable to explain why the GH values are not significantly lower in the OH group.

Despite significant lower GH in the OH group compared to the NH group, neither total nor free IGF1 were significantly different in any group despite differences in fitness or weight status. Due to the relatively high concentration of total IGF1 with low levels of GH at rest, the small reduction in total IGF1 was not statistically, nor likely physiologically significant. A lack of change in total IGF1 is in agreement with several other investigations regarding obesity and fitness in adolescents (Eliakim et al., 2006; Kamoda et al., 2005; Ubertini et al., 2008). Differences in free IGF1 of normal and obese adolescent were examined by Eliakim et al. (2006) who reported that free IGF1 of the obese subjects was less then that of the normal weight subjects however; free IGF1 was not significantly reduced. Differences were expected in the present investigation because the groups were separated by weight and fitness status, but differences were not found. The lack of a difference of free IGF1 can be partially explained by insulin, as it was significantly elevated in the OH and OL groups and correlated to free IGF1 and fitness. Insulin can reduce some of the IGF1 binding proteins (Holden et al., 1995); however the binding proteins inhibited by insulin make up only a small fraction to the total amount of binding proteins in circulation, alterations of free IGF1 are not statistically significant. Despite no significant differences in free IGF1 the slight trend for higher free IGF1 of the OL group compared to the NH group (p=0.190) may have been physiologically significant considering the increased height and amount of FFM the OL group had compared to the NH group. A post-hoc analysis correlating FFM and free

IGF1 by groups showed a significant relationship in the OL group only ($r=0.483$; $p<0.006$). The finding of no difference in free IGF1 between normal and obese children agrees with other research in adults (Nyomba et al., 1999; Ricart and Fernandez-Real, 2000).

Despite the lack of significant group differences in free IGF1 there was a significant negative association between free IGF1 and both measures of fitness. This association suggests that free IGF1 may be lower in those with high fitness levels. Given the possible influence that insulin has on free IGF1, this association makes sense. Eliakim et al. (2001) has suggested that there may be a threshold level for adiposity required to alter the GH-IGF1 axis. Perhaps if we had included overweight (85 - $<95^{th}$ percentile) as well as obese subjects ($\geq$ 95th percentile) we would have been able to better examine the effect of fitness and the influence of fitness on the GH-IGF1. However, because the OH group had non-significantly lower GH, whereas the OL group had significantly lower GH compared to the NH group, our data suggest that improved fitness may increase the BMI percentile in which GH is signaificantly affected. If this is the case, it has potential implications for the design of weight loss interventions; such as exercise programs designed to improve aerobic fitness and not soley focused on energy expenditure.

Our estimation of VO_2max is a limitation of this study. However collecting reliable VO_2max data on children in a school setting is a universal problem (Cunningham et al., 1977), especially with obese children, so estimations were used. Nevertheless our methods for predicting VO_2max has a strong correlation ($r=0.80$) to measured VO_2max (McMurray et al., 1998). The use of skinfolds to predict body fat could also be considered a limitation. However, skinfolds were measured by trained staff and done in triplicate in accordance with NHANES recommendations (1974); thus we believe the data is reliable. Finally, FFM is

admittedly not a direct measure of muscle mass. However, using FFM does eliminate fat mass and focuses more on metabolically active tissue which may better reflect the capacity of the muscle, though are results at present do not suggest using VO_2FFM versus VO_2kg matters.

In conclusion, we have demonstrated in this cross-sectional analysis that aerobically fit obese adolescents have more normal GH levels then low fit obese adolescents. However, the high levels of aerobic fitness alone do not prevent the reduction of insulin sensitivity in obesity. Our data suggests that the effect of high levels of adiposity hide much of the effect that high fitness normally may otherwise have because high fitness does not ameliorate the GH reduction entirely. It should be acknowledged that fitness may mitigate some of the reduction of GH however; strategies aimed at improving insulin sensitivity by other means may have more of an impact then improving fitness. Finally, this study demonstrates that fitness can reduce some of the obesity related GH reductions. The reduced GH level of obese low fit children has the potential to be involved in continued weight gain. Future research examining fitness level and long term changes in weight status and adiposity may provide more insights to the possible relationships highlighted by this investigation.

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CHAPTER FOUR

MANUSCRIPT TWO

Resting level of IL-6 and TNF-α in children of different weight and fitness status.

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ABSTRACT

Reports have suggested that in children aerobic fitness (VO_2max, mL/kg/min), is associated with heathlier profiles of TNF-α and IL-6; however, research to date has not accounted for differences in the adipose tissue between high-fit and low-fit individuals. The aim of the study was to examine differences in inflammatory markers of high or low fitness children who are obese or normal weight using two different oxygen uptake units, oxygen uptake per unit of fat free mass (VO_2FFM) or oxygen uptake per unit of total body mass (VO_2kg). Children (n=124; ages 8-12) were divided into four groups; normal and high-fit (NH), normal and low-fit (NL), obese and high-fit (OH), and obese and low-fit (OL). Each subject had their height, weight, skinfolds, body mass index (BMI), percent body fat, and predicted VO_2max measured. TNF-α and IL-6 levels were determined from fasting blood samples. The results showed that TNF-α was not different was between any of the groups. However, IL-6 was elevated in the NL and OL groups compared to the NH group and in the OL group compared to the OH group. TNF-α was not significantly related to fitness, but IL-6 was correlated with fitness (VO_2FFM: r=-0.356; VO_2kg: r=-0.361; $p<0.0001$). In conclusion, IL-6 levels appear to be more affected by fitness than by the amount of fat mass and the use of VO_2FFM versus VO_2kg did not alter the relationship to IL-6 or TNF-α.

INTRODUCTION

Obesity is associated with elevated levels of inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) (Maachi, et al., 2004; Caballero, 2003; van Gaal et al., 2006; Dedoussis et al., 2010; Halle et al., 2004; Hotamisligil et al., 1993). Adipose tissue is one of the main sites of production for both TNF-α and IL-6 and circulating levels of these cytokines are directly related to the amount of adipose tissue (Eder et al., 2009). From a health perspective, elevated circulating TNF-α and IL-6 are mechanisms linking obesity with insulin resistance and diabetes, as well as atherosclerosis (Eder et al. 2009; Hotamisligil et al., 1993). Thus, obesity has been characterized as a low-grade inflammatory state (Eder et al, 2009).

In contrast to obesity, increased cardiorespiratory or aerobic fitness has been associated with reduced levels of cytokines in adults (Beavers et al., 2010) and, in some way, may positively influence the relationship between obesity and cytokines. However, very little research has explored this inter-relationship in children. Although studies of children have examined the associations between fitness, fatness, obesity, and cytokines, Halle et al. (2004) was the only study found that examined the relationship of obesity and inflammation in high-fit and low-fit adolescents. These authors divided 197 children and adolescents into four groups according to BMI (<22.5 kg/m^2 = normal weight; ≥ 22.5 kg/m^2 = obese) and maximal METs (≥ 5 MET = high fit; <5 MET = low fit): obese fit, obese unfit, normal fit, normal unfit, respectively. They found that obese unfit children had elevated IL-6 compared to the other three groups. From these results Halle et al. (2004) concluded that fitness may have a positive role in reducing some of the effects of obesity on inflammation. With respect to TNF-α, unfit children, regardless of weight status had higher TNF-α levels compared to

normal or overweight fit children, suggesting that TNF-α level is primarily determined by fitness level. Thus obese adolescents with an increased level of aerobic fitness may have reduced TNF-α and IL-6 levels. However, a 5-MET (or <25 mL/kg/min) maximal capacity is low in comparison to norms for children (usually greater than 10 METs). Thus, their categorization may not represent the normal range of fitness for children.

When examining the associations between obesity related inflammation and fitness one issue of concern is the unit used to express aerobic fitness. The most common unit used is oxygen uptake relative to total body mass (ml/kg/min). However, using a unit that includes total body mass may be problematic because body mass includes both lean and fat mass. Using a unit that includes only fat free mass, such as oxygen uptake per kilogram fat free mass per minute (VO_2FFM) removes the confounding issue of fat mass in the obese and may present a more accurate depiction of the relationship between inflammation and fitness. Therefore the purpose of this study to determine if differences in TNF-α and IL-6 of adolescents exist between high and low fitness and obese and normal weight adolescents using a unit of fitness that removes the impact of increased levels of adipose tissue.

METHODS

Subjects

A total of 124 children were selected from 1486 participants in Cohort five of the Cardiovascular Health in Children III study (J.S. Harrell, P.I.), based on their body mass and fitness status. The CHIC III study was an investigation of youth from rural North Carolina exploring risk factors for cardiovascular disease and metabolic syndrome. Of the original 1486 participants, the sex and race distribution was ~50% male, 50% female, 55% African American, 37% Caucasian, and 8% other races, with a mean age was 9.7±1.1 years old. The

subsample used for the present investigation included subjects aged 10.0 ± 0.9 (52 female, 72 male, 72 African American, 39 Caucasian, and 13 other races), split into 4 groups based upon their weight and fitness (VO_2peak) status: obese high-fit (OH), obese low-fit (OL), normal weight high-fit (NH), and normal weight low-fit (NL). Only adolescents in Tanner stage 1-3 were included in the study. All subjects gave written assent and their parents provided consent before participation, by signing the University of North Carolina at Chapel Hill IRB approved forms.

Data collection procedures

All anthropometric and exercise testing data was collected in the subject's school during the school day, with the exception that blood was drawn early in the morning. Height was measured using a standard calibrated stadiometer (Perspective Enterprises, Portage, MI). Body mass was determined using a calibrated electronic scale (Model 2101L, Healthometer Medical, Bridgewater. IL). Body mass index (BMI) was calculated: [weight (kg)/height $(m)^2$]. Pubertal status was estimated using the Pubertal Development Scale (Petersen et al., 1988), a sex-specific self-administered questionnaire with 5-item subscales. Skinfolds were measured in triplicate using calibrated Lange Skinfold calipers (Cambridge Scientific, Cambridge, MD) from the subscapula and triceps (NHANES III, 1974). These measurements were used to estimate body fat percentage using sex, race, and age based formulas (Slaughter et al., 1988).

Aerobic power (VO_2max) was estimated from a three stage submaximal cycle ergometer test using the methods of McMurray et al. (1998). This method has been shown to produce correlations as high as $r = 0.80$ with measured VO_2max. The cycle ergometers used

in testing were a BodyGuard (model 990), Tunturi magnetic-braked Tunuri Oy Ltd., (Turku, Finland), or Monarch (model 818; Monark, Varberg, Sweden) cycle ergometer.

Group Determination

Weight status was determined based upon the Centers for Disease Control and Prevention (CDC) growth charts from the year 2000 (CDC, 2000). Normal weight was defined as $<85^{th}$ and $>5^{th}$ BMI percentile for age and sex. Obese was defined as $\geq 95^{th}$ BMI percentile for age and sex. The VO_2max per kilogram of the fat free mass (VO_2FFM) was determined from estimations of body fat percentage and absolute VO_2max (mL/min). Fat free mass (FFM) was determined by subtracting predicted body fat percentage from one and multiplying by the subjects total body mass (kg). Finally, absolute VO_2max (mL/min) was divided by FFM giving the unit mL of O_2 per kilogram of fat free mass per min ($mL/kg_{FFM}/min$) or VO_2FFM. For comparison with previous literature, VO_2max was also computed in units of mL of oxygen per kilogram body mass (VO_2kg).

Fitness was determined based upon predicted VO_2FFM which was used in place of VO_2max of the total body mass (mL/kg/min). Fitness cut-points were developed (unpublished data) based on data from CHIC cohorts I, II, and III, collected from 1992-2005, representing 3235 adolescents from age 8-12. Subjects that were in the 66^{th} percentile or above for VO_2FFM were included in the high fitness group; those with VO_2FFM less then the 33^{rd} percentile were included in the low fitness group. Cutoffs by age and sex are reported elsewhere (manuscript 1; Tables 1 and 2; page)

All subjects in the original study fitting both the obese and high fitness criteria with complete data were included in the OH group. Potential subjects in the other three groups were determined based upon fitness and weight status. Selection into the NH, NL, or OL

group was done matching sex and pubertal status to the OH group. Of the original 1486 subjects, 44 reached the criteria of obese and highly fit but only 31 of these had complete data and blood samples. Subjects for the remaining groups were chosen by random selection after meeting group criteria for weight and fitness status and matching for age and pubertal status with subjects in the OH group.

Blood analysis

Subjects were called the day before blood draws were to occur and reminded to not eat anything and drink only water before their blood was draw. Upon arrival subject's confirmed their overnight fast. The antecubital space of the arm of the subject's preference was used for all blood draws. The blood draw samples were centrifuged, separated into individual mircocentrifuge tubes with ~0.5 ml sample per tube, then placed on dry ice to be transported to our storage facility where they were kept at -80˚C until analysis.

All blood analysis was completed on stored samples, using commercially available assay kits. Serum IL-6 values were determined using ELISA technique (Invitrogen, Camirillo, CA, USA). The intra-assay coefficient of variation (CV) for IL-6 was 7.4%; with aninter-assay CV of 9.9%. Invitrogen reports a sensitivity of <0.09 pg/mL. Tumor necrosis factor α (TNF-α) values were also measured using an ELISA technique (Thermo Scientific, Rockford, IL, USA). The sensitivity of the TNF-α assay is <1 pg/mL. The intra-assay CV for TNF-α was 4.1%; with an inter-assay CV of 11.2%.

Statistical analysis

Means and SEM were computed for all variables by group (NH, NL, OH, and OL). To determine if differences existed between the groups 2x2 analysis of variance (ANOVA) was conducted separately for IL-6 and TNF-α. When an ANOVA analysis was significant

($p<0.05$), tukey post-hoc test was applied to determine which groups were different. To further explore any inter-relationships, Spearman correlations were performed between TNF-α, IL-6, and measures of fitness (VO_2FFM and VO_2kg). The Spearman correlations were also performed after controlling for differences in body fat percentage. Spearman correlations were used because normality of our measures cannot be assumed because of the polarilty involved in the group selection. The alpha level was set at $p<0.05$. All statistical analysis was computed using SAS version 9.1 (Cary, NC).

RESULTS

Table 1. Mean ± standard deviation of anthropometric and fitness variables presented by group

	Normal High Fitness	Normal Low Fitness	Obese High Fitness	Obese Low Fitness
N	31	31	31	31
Age (yrs)	9.9±0.9	10.1±1.0	10.1±0.8	10.0±0.9
Tanner Stage	2.2±0.7	2.3±0.7	2.3±0.7	2.3±0.7
Height (cm)	139±8.1†‡	143±9.3	147±9.4*	148±9.1*
Body Mass (kg)	33.9±5.2†‡	36.7±6.5†‡	60.9±17.4*#	61.2±11.2*#
BMI (kg/m^2)	17.4±5.7†‡	17.6±1.7†‡	27.7±4.9*#	27.7±3.4*#
BMI percentile	53.2±20.8†‡	56.8±24.0†‡	97.8±1.5*#	98.1±1.2*#
Fat percentage	17.4±5.7†‡	16.0±6.6†‡	36.7±11.0*#	32.2±7.3*#
Fat Free Mass (kg)	27.9±4.1†‡	30.8±5.4†‡	37.1±7.4*#	40.9±6.0*#
VO_2FFM	62.1±8.1‡#	35.4±6.6†*	64.8±10.3‡#	35.1±6.6†*
VO_2kg	51.3±7.5#†‡	29.8±6.4*†‡	40.8±9.0*#‡	23.9±5.6*#†

* $p<0.05$ from NH, # $p<0.05$ from NL, † $p<0.05$ from OH, ‡ $p<0.05$ from OL

Complete results for the group characteristics can be found in Table 1. Each group had 13 females and 18 males and groups did not differ by age or Tanner stage. The OH and OL groups had significantly greater height, body mass, BMI, BMI percentile, fat percentage,

and fat free mass compared to the NH group (see Table 1). Similar results were found for the obese groups compared to the NL group, with the exception of height as both obese groups were significantly taller then the NL group. The VO_2FFM was higher in both the NH and OH groups compared to the NL and OL groups. All groups were significantly different from one another in regard to VO_2kg ($p < 0.05$). The NH group had the highest VO_2kg, followed by the OH and NL, with the OL group having the lowest VO2kg.

Results for IL-6 and TNF-α can be found in Figures 1 and 2, respectively. Resting IL-6 levels in the OL group were significantly elevated compared to the OH and NH ($p<0.05$) groups. The NL groups resting IL-6 levels were significantly elevated compared to the NH group ($p<0.05$). No statistically significant group differences were noted for TNF-α. However, a trend was found between the OL and NH groups differences ($p=0.057$; Figure 2).

Figure 1. Mean (±SEM) resting IL-6 level of the normal weight high-fit (NH), normal weight low-fit (NL), obese high-fit (OH), and obese low-fit (OL) groups.

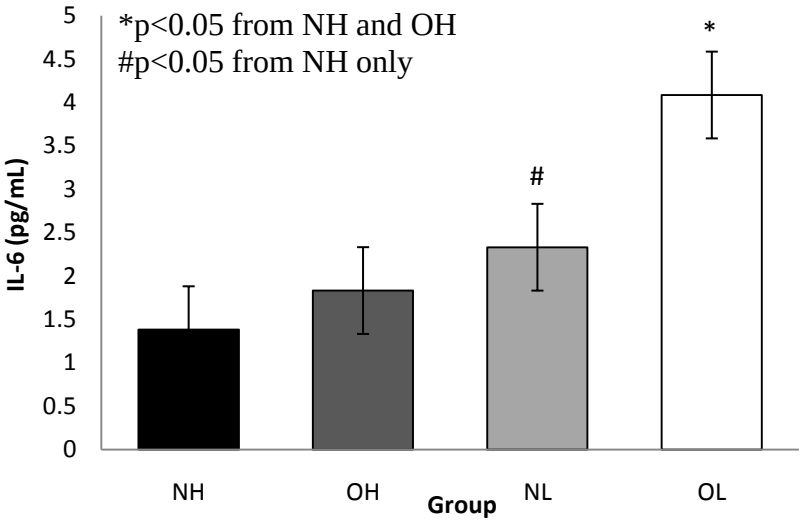
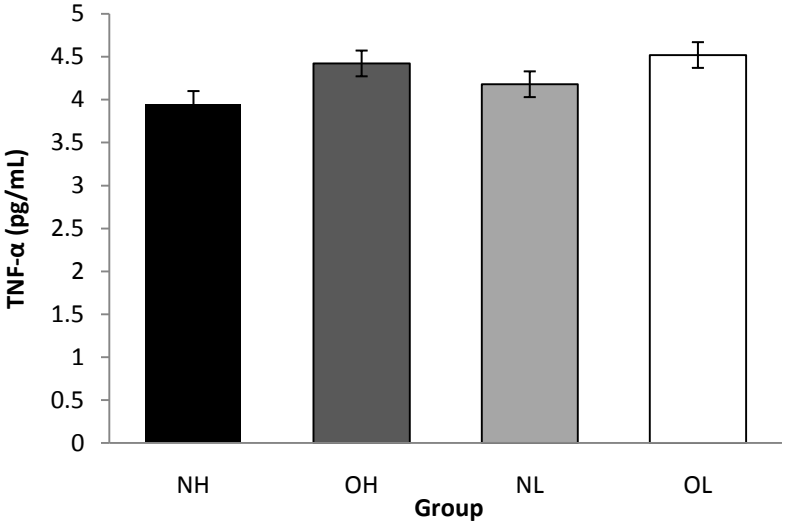


Figure 2. Mean (±SEM) resting TNF-α level of the normal weight high-fit (NH), normal weight low-fit (NL), obese high-fit (OH), and obese low-fit (OL) groups.



Result of the Spearman correlation between IL-6 and fitness measures revealed significant negative relationships (Table 2). No significant relationships were found between the fitness measures and TNF-α

Table 2. Spearman correlations for TNF-α and IL-6 between VO_2 of the fat free mass (VO_2FFM) and VO_2 of the total body mass (VO_2kg).

	VO_2FFM	VO_2kg
TNF-α	-0.138	-0.145
IL-6	**-0.356***	**-0.361***

*p=0.0001

DISCUSSION

This study compared normal weight adolescents of high or low fitness to obese adolescents of high or low fitness to examine the effect that fitness and fatness can have on markers of inflammation. In addition, the fitness unit VO_2FFM was used to remove the potential confounding effect that large amounts of adipose tissue has on the relationships

between inflammation, fitness and fatness. The findings show that higher levels of fitness are associated with lower levels of IL-6, independent of obesity. Finally, TNF-α was not different between the groups; however, a strong trend did exist between the NH and OL groups ($p = 0.057$), suggesting a potential influence of TNF-α that may be more similarly affected by fitness and obesity.

The relationship between obesity and inflammation has been well established (Hotamisligil et al., 1993; Eder et al., Weisburg et al., 2003); however, much less attention has focused on the effect that fitness may have on this relationship (Halle et al., 2004). The findings of this investigation suggest IL-6 was more related to fitness, whereas the results of Halle et al. (2004) suggest that TNF-α is more related to fitness. One possible explanation for these divergent findings could be the group determination. Halle et al. (2004) separated their groups based on BMI with normal weight being defined as a BMI < 22.5 kg/m^2 and obesity defined as BMI ≥ 22.5 kg/m^2. Based on the age and gender of the Halle subjects, the BMI percentile for inclusion in the obese group would have been between the 76th and 95th percentile; thus some subjects in the "obese" group were not obese based on the CDC definition of obesity. Including non-obese subjects in an "obese" group posses a potential problem because alterations to resting hormonal levels that influence inflammation occur around 30% body fat (Considine et al., 1996) or the 85th BMI percentile (Eliakim et al., 2001). Halle's "obese" subjects would have been overweight not obese. Futher, Halle et al. (2004) defined high-fit adolescents as having a maximal MET capacity ≥ 5 METs and low-fit adolescents have a maximal MET capacity < 5 METs; meaning children with a maximal MET capacity of just over 5 METs would have been grouped in the high fit group. Possessing a maximal MET capacity of even 6 would still be very low, for instance when

estimating our low fit subjects MET capacity based on there average maximal VO_2kg, they had an average maximal MET capacity of 8.5 and 6.5 for the normal and obese weight group, respectively. Therefore, it is likely that some of subjects in the Halle study considered high fit were not actually high fit. Further, the grouping pattern used by Halle did not separate what was considered normal versus obese or high versus low fitness, whereas the present investigation used polarized groups to allow for distinct differences. Without polarization of the groups, inclusion of overweight individuals in either group is likely to have occurred, which may have clouded the results of Halle et al. (2004). In the present investigation groups clearly had different characteristics and thus may give a clearer picture of the potential differences found. Therefore, it is believed the conclusion that IL-6 levels are more associated with fitness level is appropriate.

The trend for higher TNF-α levels in the OL group may help explain some of the elevation in IL-6, since TNF-α is known to stimulate IL-6 (Fasshauer et al., 2003; Willerson and Ridker, 2004). Fasshauer et al. (2003) showed that TNF-α may stimulate IL-6 release through autocrine/paracrine mechanisms when adipocytes treated with TNF-α saw IL-6 increase 74-fold in vitro. However, the finding that TNF-α was not significantly elevated despite increased IL-6 is supported by the findings of Russell et al. (2009). They found TNF-α receptors to be elevated in obese adolescent girls, which would allow TNF-α to bind to its receptor more easily and have a greater effect (such as IL-6 stimulation) without a need for increased circulating TNF-α. Further, if our adolescents experienced a similar increase in TNF-α acting via autocrine/paracrine mechanisms or TNF-α receptors that would help defend the increase in IL-6 with only slightly higher levels of TNF-α in the OL group. However, a difference in TNF-α of approximately 0.5 pg/mL may not be physiologically

significant but the potential increase in TNF-a receptors found in many tissues, including adipose tissue (Bazzoni and Beulter, 1996), may be what allowed for the increased IL-6 levels in the present investigation. Also, IL-6 was elevated in the NL group, but not in the OH group, which further suggests that elevated fitness can allow for lower IL-6 levels independent of obesity.

The present investigation included obese, not overweight, adolescents that had BMI's at or above the 95^{th} percentile. Such a BMI typically represents clinical obesity with large amounts of fat mass. In addition, many of the alterations that occur in obesity are enhanced as fat mass increases (Dedoussis et al., 2010; Eliakim et al., 2001; Martin et al., 2005). Yet, based on the lack of association between TNF-α or IL-6 and BMI in our post hoc correlations it is possible that by grouping our subjects into categories, and not using a continuum of individuals, hindered the ability to explore associations within the combined groups; hence, the reason no relationship between TNF-α or IL-6 and BMI was evident.

VO_2max and body fat percentage were estimated which is a limitation of the present investigation. However collecting reliable VO_2max data on children in a school setting is a universal problem (Cunningham et al., 1977), especially obese children, for this reason estimations were used. Data collection occured in schools, which prevented us from being able to transport the necessary equipment to gather more precise physiological measurements. However, our methods for predicting VO_2max was strong (r=0.80; McMurray et al., 1998) and skinfolds were measured in triplicate by trained staff using NHANES procedures (1974). Finally, FFM is admittedly not a direct measure of of muscle mass. However, using FFM does eliminate or reduce fat mass and focuses more on

metabolically active tissue which may better reflect the capacity of the muscle, though are results at present do not suggest using VO_2FFM versus VO_2kg matters.

CONCLUSION

The findings of the present study show relationships between inflammatory markers and fitness were not different when using either unit (VO_2FFM or VO_2kg) probably because inflammation was not related to aerobic fitness. However, aerobic fitness was associated with lower levels of inflammation in obesity, particularly IL-6 level; whereas TNF-α may be minimally affected by fitness and obesity. Future research should explore the degree to which obesity and fitness influence autocrine/paracine action of TNF-α and TNF-α receptors to determine if these are influencing IL-6 as seen in the present investigation.

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CHAPTER FIVE

MANUSCRIPT THREE

The influence of TNF-α and IL-6 on the relationship between components of the GH-IGF1 axis of adolescents from a wide range of fitness and weight status

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ABSTRACT

Obesity is generally accompanied by increased circulation of tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) which can influence the function of the growth hormone - insulin-like growth factor 1 axis (GH-IGF1). Obese individuals with increased fitness may have lower levels of inflammatory cytokines compared to obese non-fit individuals allowing for less obstruction of the GH-IGF1 axis and greater ability to maintain weight. Therefore this study examined the influence of inflammatory markers on the relationship between components of the GH-IGF1 axis of adolescents with wide range of fitness and weight status. Children, ages 8-12 (n=124) had their height, weight, skinfolds, body mass index (BMI), and body fat % were computed, and VO_2max predicted. Levels of TNF-α, IL-6, GH, total IGF1, free IGF1, and insulin were determined from a morning resting blood sample. Growth hormone was significantly correlated to VO_2max expressed per unit mL/kg/min (VO_2kg; r = 0.233) and percent body fat (r = -0.208). Free IGF1 was significantly correlated with VO_2max expressed per unit fat free mass (VO_2FFM; r= -0.243), VO_2kg (r = -0.300), body fat (r = -0.293), and insulin (r = -0.338). Total IGF1 was not correlated with fitness or fatness. Significant correlations existed between GH and total IGF1 (r = 0.194, p = 0.05) and free IGF1 and total IGF1 (r = 0.607, p < 0.001). IL-6 did not contribute to the relationship between GH and Total IGF1. IL-6 had a significant inverse relationship between free IGF1 and total IGF1 when fitness was included in the model. Results suggest that IL-6 does not influence the GH:total IGF1 relationship but can influence the free IGF1:total IGF1, when fitness is included in the regression model.

INTRODUCTION

The growth hormone – insulin-like growth factor 1 (GH-IGF1) axis consists of growth hormone (GH) released from the pituitary which stimulates insulin-like growth factor 1 (IGF1) release from the liver. Physiological functions of the GH-IGF1 axis include muscle development and hypertrophy, body composition changes, bone mineral density and cognitive functioning (Alexopoulou et al., 2010; de Bie et al., 2010). Inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) have been shown to modify the GH-IGF1 axis by interfering with both normal GH release and intracellular IGF1 signaling which may negatively impact muscle growth (Aguirre et al., 2000; Gao et al., 2002; Rui et al., 2002). Obese individuals typically have increased levels of circulating TNF-α and IL-6 (Maachi et al., 2004; Caballero, 2003; van Gaal et al., 2006). Thus a reduction in muscle mass might be expected in obese individuals, yet the opposite is typically found (Freedman et al., 2005). A possible explanation for the increased muscle mass with elevated inflammation may be that resting insulin levels known to be elevated in obesity can lead to increased free or bioactive IGF1 (Nyomba et al., 1997).

Obesity is associated with increased inflammation, yet cardio-respiratory or aerobic fitness has been associated with reduced levels of inflammation (Beavers et al., 2010). Thus, fitness may in some way positively influence the relationship between obesity and cytokines. There is limited work exploring the inverse relationship between fitness and inflammation in obese adolescents (Halle et al., 2004; Rubin et al., 2008). Halle et al. (2004) separated children and adolescents into obese high-fit, obese low-fit, normal high-fit, normal low-fit groups according to BMI and maximal metabolic equivalent (MET) capacity. The results indicated that obese low-fit subjects had increased IL-6 compared to the other three groups.

The high-fit children, regardless of weight status had lower levels of TNF-α level than low-fit children. The authors concluded that the subject's fitness level contributed to a more favorable cytokine profile, even in obese children. Rubin et al. (2008) looked at the association of predicted maximal oxygen uptake and TNF-α and found a significant association in the girls but not the boys. Neither study controlled for differences in body fat that may have impacted their measures of fitness.

The problem of adolescents transitioning from overweight to obese is on the rise (Ogden et al., 2010). The transition from overweight to obese may be related to shifts in metabolically related endocrine markers. Alterations to the GH-IGF1 axis are a result of the weight gain, not the cause (Rasmussen et al., 1995); thus, minimizing the alterations to the GH-IGF1 axis such as reduced resting and stimulated GH levels (Eliakim et al., 2006; Nam and Marcus, 2000) which stimulate fatty acid oxidation (Leroith and Yakar, 2007; Vijayakumar et al., 2010) and could help prevent continued weight gain. Physical fitness, which may reduce levels of TNF-α and IL-6 may also allow for more normal GH-IGF1 axis functioning and mitigate further weight gain as well. One way to study these complex interactions in children is to sample individuals of differing levels of both weight status and fitness level. Such studies may be able to separate some of the interactions between fitness and fatness and provide a rational for further study into the explanation of competing signals. The present cross-sectional investigation examined the influence of inflammatory cytokines TNF-α and IL-6 on the GH-IGF1 axis in adolescents of varying weight and fitness status. The purpose was to determine if accounting for differences in circulating TNF-α or IL-6 alters the relationship between GH, free IGF1 and total IGF1.

METHODS

Subjects

Subjects were obtained from the Cardiovascular Health in Children III (CHIC III) study, Cohort 5 (J.S. Harrell, P.I.). The CHIC III study was designed to investigate cardiovascular health risk factors for North Carolina's rural youth. The mean age of the original 1486 participants was 9.7±1.1 years old, with an even sex distribution (~50% male, 50% female) and a race distribution of 55% African American, 37% Caucasian, and 8% other race. The University of North Carolina at Chapel Hill IRB approved consent (parents) and assent (child) forms were signed prior to participation in the study. In the present investigation, 124 children and adolescents (72 female, 52 male, 72 African American, 39 Causasian, and 13 from other races) were selected from the larger subject pool and included an age range of 8-12 years. Subjects with a Tanner stage of ≤ 3 were selected based on their individual weight and fitness status in order to have equal representation of subjects who were normalweight high-fit, normal weight low-fit, obese high-fit, and obese low-fit.

Data collection procedures

Data collection took place in the subject's school during the school day. Blood samples were also collected at the schools in the morning following an overnight fast. Height and body mass were measured using a stadiometer (Perspective Enterprises, Portage, MI) and calibrated electronic scale (Model 2101KL, Healthometer Medical, Bridgewater, IL), respectively. Body mass index (BMI) was calculated: weight (kg)/height $(m)^2$. Triceps and subscapular skinfolds were measured in triplicate (NHANES III, 1974) using calibrated Lange skinfold calipers (Cambridge Scientific, Cambridge, MD). Skinfolds were used to estimate body fat percentage using sex, race, and age formulas (Slaughter et al., 1988). Fat

free mass (FFM) was determined by subtracting estimated body fat percentage from one and multiplying by the subjects total body mass (kg).

Aerobic power (VO_2max) was estimated using heart rate response to a known workload on a cycle ergometer using PWC_{195} methodology (McMurray et al., 1998). This method has produced high correlations (r = 0.80) with measured VO_2max using units relative to total body mass. All ergometers [BodyGuard (model 990), Tunturi magnetic-braked Tunuri Oy Ltd., (Turku, Finland), or Monarch (model 818; Monark, Varberg, Sweden)] were calibrated immediately before each day of testing. Polar Pacer heart rate monitors, calibrated against an electrocardiogram, were used to measure heart rate. VO_2max per kilogram of the fat free mass (VO_2FFM) was determined from estimations of body fat percentage and absolute VO_2max (mL/min).

Subjects were selected based on weight and fitness status and included equal representation from subjects who were obese low-fit, obese high-fit, normal weight high-fit, normal weight low-fit. The Center for Disease Control (CDC) definitions were used to define our subjects as normal weight and obese (CDC, 2000). These definitions describe normal weight was as $<85^{th}$ and $>5^{th}$ BMI percentile for age and sex, whereas obese is defined as $\geq 95^{th}$ BMI percentile for age and sex. Overweightchildren and adolescents, that is, those witha BMI percentile of ≥85 and <95 were not included. Criteria for the fitness level of the subjects was determined based upon estimated VO_2max of fat free mass (VO_2FFM). Subjects with a VO_2FFM less then the 33^{rd} percentile were included in the low fitness group; those with a VO_2FFM greater than the 66^{th} percentile were included in the high fitness group. Subjects from the different categories were additionally matched based on gender and pubertal status. Subjects were match based on pubertal status and limited to

Tanner stage 1-3 in order to reduce any gender differences in development which have been shown to potentially influence the fitness measure VO_2FFM (Janz et al., 1998). For a complete description of subject determination see manuscript 1.

Blood analysis

Subjects were called the day before and reminded not to eat anything after bedtime and drink only water until after the blood draw. Upon arrival the next morning subjects were asked to confirm their overnight fast. Blood samples were obtained using the antecubital space of the arm of the subject's preference. All samples were immediately centrifuged and plasma or serum separated into individual mircocentrifuge tubes (~0.5 mL plasma or serum per tube). Samples were then placed on dry ice and transported to the Applied Physiology Laboratory on the campus of the University of North Carolina, where they were stored at -80˚C until analysis.

All blood analysis was completed on stored plasma or serum samples in duplicate, unless otherwise stated, using commercially available assay kits. Serum total GH values were determined using ELISA technique (IBL-America, Minneapolis, MN). The CV for GH was 6.1%; with an inter-assay CV of 4.0%. The sensitivity of the GH assay was 0.2 ng/mL as reported by IBL-America. Total IGF1 (total IGF1) values were measured using ELISA technique (R&D System Laboratories, Minneapolis, MN). The sensitivity of the total IGF1 assay is reported as 0.026 ng/mL. The intra-assay CV for total IGF1 was 3.7%; with an inter-assay CV of 4.1%. Free IGF1 (free IGF1) concentration was determined in EDTA plasma using ELISA technique (Diagnostics Systems Laboratories, Webster, TX) and had a reported sensitivity of 0.015 ng/mL. The intra-assay CV for free IGF1 was 4.3%; while the inter-assay CV was 10.2%. Serum IL-6 values were determined using ELISA technique

(Invitrogen, Camirillo, CA, USA). The intra-assay coefficient of variation (CV) for IL-6 was 7.4%; with and inter-assay CV of 9.9%. Invitrogen reports a sensitivity of <0.09 pg/mL. Tumor necrosis factor α (TNF-α) values were also measured using an ELISA technique (Thermo Scientific, Rockford, IL, USA). Thermo Scientific reports the sensitivity of the TNF-α assay to be <1pg/mL. The intra-assay CV for TNF-α was 4.1%; with an inter-assay CV of 11.2%. Plasma insulin levels were determined using a commercially available kit (Linco, St. Charles, Mo., USA). Linco reports 0.02% cross-reactivity with proinsulin, glucagon, somatostatin, IGF-1 or pancreatic polypeptide. Our intra-assay coefficient of variation (CV) between duplicate samples was less than 8%.

Statistical analysis

The purpose of this investigation was to explore how inflammatory markers can affect the inter-relationships between GH-IGF1 axis components across varied levels of fitness and fatness. Spearman correlations were first computed between components of the GH-IGF1 axis (GH, total IGF1, and free IGF1) to determine which associations had significant relationships and warranted further exploration. To determine which variables to include in the regression analysis Spearman correlation coefficients were then calculated between GH, total IGF1, and free IGF1 and VO_2FFM, VO_2KG, body fat, and insulin. Spearman correlations were run because subjects were not normaly distributed. Variables that were significantly correlated to GH, total IGF1 and free IGF1 were added to multiple regression models in a stepwise fashion to determine if they influenced to relationship between GH-IGF1 components and inflammatory markers. The alpha level was set at $p<0.05$. All statistical analysis was computed using SAS version 9.1 (Cary, NC).

RESULTS

The mean age of our subjects was 10.0 ± 0.7 years and mean pubertal status was 2.3 ± 0.7. Fitness and fatness variable ranges for all subjects are presented in Table 1.

Table 1. Ranges of fitness and fatness variables

Fat Free Mass (kg)	18.2 – 53.6
Fat percentage (%)	5.0 – 83.6
BMI (kg/m^2)	14.7 - 43.5
BMI percentile	6.6 - 99.8
VO_2kg (mL/kg/min)	13.9 – 68.1
VO_2FFM (mL/kg_{FFM}/min)	22.6 – 90.5

Spearmen correlations revealed significant relationships between GH and total IGF1 ($r=0.190$, $p=0.03$) and free IGF1 and total IGF1 ($r=0.603$, $p<0.0001$), but not for GH and free IGF1 ($r=0.129$, $p=0.15$). Thus, further regression analysis to examine the influence of inflammatory markers included only the significant relationships between GH and total IGF1 and free IGF1 and total IGF1.

Table 2. Spearman correlations between growth hormone, total IGF1, or free IGF1 and VO_2FFM, VO_2KG, body fat, and insulin.

	Growth Hormone	Total IGF1	Free IGF1
IL-6 (pg/mL)	-0.100	0.213*	0.096
TNF-α (pg/mL)	-0.113	-0.114	-0.079
VO_2FFM (mL/kg_{FFM}/min)	0.150	-0.161	-0.243*
VO_2kg (mL/kg/min)	0.233*	-0.154	-0.300*
Body Fat (%)	-0.208*	0.128	0.293*
Insulin (pmol/L)	-0.099	0.182	0.338*

*$p<0.05$

Growth hormone was not related to IL-6 but was significantly correlated with VO_2kg and body fat. See Table 2. Total IGF1 was significantly related to IL-6 only, whereas free IGF1 was significantly related to VO_2FFM, VO_2kg, body fat, and insulin. TNF-α was not significantly related to GH, tIGF, or free IGF1; therefore, TNF-α was omitted from further

analysis and only IL-6 was used to explore if inflammation influences significant relationship between GH and between total IGF1 and free IGF1 and total IGF1.

Table 3 includes results of the regression analysis between GH and total IGF1. The relationship between GH and total IGF1 was not significant (p=0.437). The addition of IL-6 to the model did not significantly impact or alter the relationship, as the total R^2 only increased from 0.005 to 0.010. Finally, when VO_2kg and body fat were added, thus including all variables that were significantly related to either GH or total IGF1, the model only explained a small percentage of the variance and none of the variables contributed significantly.

Table 3. Regression analysis for the relationship between growth hormone, total IGF1 and IL-6 and correlated variables VO_2kg and body fat.

		Growth Hormone		
Step		Total R^2	β-weight	p-value
1	Total IGF1	0.005	0.000	0.437
2	Total IGF1	0.010	0.001	0.451
	IL-6		0.039	0.551
3	Total IGF1	0.068	0.001	0.277
	IL-6		0.036	0.348
	VO_2kg/min		0.012	0.222
	Body fat		-0.016	0.088

Results of the regression analysis between free IGF1 and total IGF1 are found in Table 4. Free IGF1 and total IGF1 were significantly related; adding IL-6 alone increased the total R^2 from 0.424 to 0.462, but did not significantly contribute to the relationship (p=0.193). VO_2FFM significantly contributed to the model (p=0.015), but only increased the total R^2 from 0.424 to 0.439. When both IL-6 and VO_2FFM were added the total R^2 increased to 0.492 and both variables significantly contributed to the model (IL-6, p=0.046 and VO_2FFM, p=0.012). Adding IL-6, VO_2FFM, body fat, and insulin with free IGF1 only produced a total R^2 of 0.525. When VO_2kg was used instead of VO_2FFM the total R^2 was

only increased from 0.424 to 0.512 and the remaining variables had similar β weights and p values; thus those results are not presented.

Table 4. Regression analysis for the relationship between free IGF1, total IGF1 and IL-6 and correlated variables: body fat, VO_2FFM and insulin.

		Free IGF1		
Step		Total R^2	β weight	p
1	Total IGF1	0.424	0.008	<0.001
2	Total IGF1	0.462	0.008	<0.001
	IL-6		-0.036	0.193
3	Total IGF1	0.439	0.008	<0.001
	VO_2FFM/min		-0.009	0.015
4	Total IGF1	0.492	0.007	<0.001
	IL-6		-0.051	0.046
	VO_2FFM/min		-0.012	0.012
5	Total IGF1	0.525	0.007	<0.001
	IL-6		-0.063	0.020
	VO_2FFM/min		-0.017	0.007
	Body Fat		0.027	0.021
	Insulin		-0.011	0.128

DISCUSSION

In the present investigation the influence of inflammatory markers, IL-6 and TNF-α on alterations of the GH-IGF1 axis in adolescents was explored using a wide range of weight and fitness status. Associations of GH-IGF1 axis components; GH, free IGF1, and total IGF1 showed GH is associated with total IGF1, similarly free IGF1 is associated with total IGF1. IL-6 contributed to the relationship between free IGF1 and total IGF1 when fitness is included in the regression equation (Table 4). However, IL-6 did not appear to influence the relationship between GH and total IGF1 (Table 4) nor did TNF-α appear to be related to alterations of the GH-IGF1 axis (Table 3). These findings suggested that IL-6 impacts the relationship between free IGF1, through possibily some synergistic relationship with fitness (VO_2FFM or VO_2kg). While the exact mechanism that explains the relationship between

fitness, IL-6 and free IGF1 are not known, this finding may represent an explaination for normal levels of IGF1 in spite of reduced GH found in obesity (Frystyk et al., 1995).

To the best of our knowledge this is the first study to examine the effect of fitness and fatness on the association between inflammation and the GH-IGF1 axis in children. Previous work examining fitness, fatness and inflammation noted that children with higher fitness had lower levels of the inflammatory markers IL-6 and TNF-α compared to children of lower fitness level independent of fatness (Halle et al., 2004). In addition, higher levels of fitness have been associated with increased GH in normal weight adolescents (Eliakim et al., 1996; Ubertini et al, 2008). None of these studies addressed all four issues (fitness, fatness, cytokines, and the axis) simultaneously. In the present investigation, while the directionality of the relationship agrees with previous research, the results were not statistical significant. The lack of significance in the present study may be related to differences in subject populations. Neither Eliakim et al. (1996) nor Ubertini et al. (2008), purposefully included a wide range of fitness or fatness levels. In fact, subjects in the Ubertini et al. (2008) were athletes with an age range of 16-26 years and subjects in Eliakim et al. (1996) were all females and between the age of 15-17 as opposed to the present study that used both genders who were 8-11 years old. Therefore, results suggest a possible relationship between GH and fitness, but this relationship does not appear to be related to IL-6 or TNF-α.

The rationale for a significant correlation existing between GH and total IGF1 is appropriate because GH is the stimulating hormone for IGF1 release in the liver (Clemmons, 2007). The Spearman correlation was weak but significant ($r=0.190$, $p=0.03$). However, when examined through simple regression the results were no longer significant ($R^2=0.005$ $p=0.437$). Our results agree with the findings of Eliakim et al. (1998), who found that

adolescent boys with a higher level of fitness had elevated GH but no difference in tIGF-1. These authors suggest that the fitter males had reduced hepatic sensitivity to GH (Eliakim et al., 1998).

A significant relationship between the cytokines and GH-IGF1 axis was expected since IL-6 inhibits GH action in hepatic tissue, which in turn influences the release of IGF1 (Tan et al. 2005). Data from the present study does not suggest that IL-6 impacts this relationship, as adding IL-6 to the regression equation did not influence the relationship, nor did adding VO_2kg or body fat. IL-6 does not appear to impact the relationship between GH and IGF1 when fitness (VO_2kg) is accounted for even though IL-6 has been shown to be affected by fitness level independent of obesity (Hosick et al.; manuscript 2). Thus we conclude that IL-6 has minimal influence on the relationship between GH and total IGF1 even when accounting for different levels of fitness.

The Spearman correltion between free IGF1 and total IGF1 was of moderate strength ($r=0.607$, $p<0.001$), as was the simple linear regression model ($R^2=0.424$, $p<0.001$; Table 4). These findings are in agreement with the results of previous research using similar populations (Bareket et al., 1996; Juul et al., 1996). When IL-6 was added to the regression model IL-6 alone did not significantly contribute to the model. However, when VO_2FFM was included in the model the influence of IL-6 was significant and inversely related suggesting the relationship between IL-6 and free IGF1 and total IGF1 is somehow dependant upon VO_2FFM. Of interest is the inverse relationship (IL-6; $\beta = -0.051$) that was found between IL-6 and free IGF1. Free IGF1 has been found to be elevated in obesity as is IL-6 (Fried et al., 1998; Shoelson et al., 2006); thus a direct relationship was expected. The inverse relationship may be explained by the weight and fitness grouping of our subjects, as

fitness has been associated with lower IL-6 independent of obesity (Halle et al., 2004; manuscript 1). Thus, the inverse relationship between fitness and IL-6 may have allowed for an inverse relationship between IL-6 and free IGF1 based on our subject population; however, further research is required to test this hypothesis. If IL-6 is more related to fitness than obesity the source of the majority of IL-6 comes into question, especially in non-obese children with low fitness.

These findings suggest that fitness influences the relationship between free and total IGF1 because when fitness was accounted for IL-6 independently contributed to the relationship between free and total IGF1. However, these results are to be interpreted cautiously as subjects were chosen based on their weight and fitness status and thus included equal numbers of normal high-fit children and obese high-fit. Yet, of the 1486 potential subjects in this study 198 were normal high-fit, whereas 42 were high-fit obese; therefore, our results may not be representative of the general population based on over representation of obese high-fit subjects.

Both the Spearman correlational and linear regression analysis revealed a significant relationship between total IGF1 and free IGF1. However, particularly in the regressions, IL-6 required fitness to be included to be significant. Further, both analyses explained less then 50 percent of the varience in our measurement. Thus, in an attempt to further understand how the relationship between free and total IGF1 is influenced by changes in fitness and IL-6 individually the free/total IGF1 ratio was calculated. The free/total IGF1 ratio has been used as an indication of growth or training condition (De Palo et al., 2008; Yamada et al., 1998). To analyze the change in the free/total IGF1 ratio, which tended to increase as level of obesity increased, Spearman correlations were run between the ratio and body fat, VO_2FFM

and IL-6. The free/total IGF1 ratio had a significant positive association with body fat ($r=0.264$, $p<0.01$), a negative relationship with VO_2FFM ($r=-0.221$, $p=0.014$), and no significant relationship with IL-6 ($r=0.036$, $p=0.701$). The positive relationship with body fat would agree with Attia et al. (1998) who found non-obese adolescents had significantly elevated total IGF1 but slightly lower free IGF1 values compared to obese adolescents. Attia et al. (1998) suggest their findings indicate obese alterations in the GH-IGF1 serve to increase free IGF1 and contribute to other alterations seen in the GH-IGF1 axis of obese individuals. In addition, increased linear growth in obese adolescents would agree with the positive relationship as the obese children would have elevated amounts of bioactive free IGF1 (Yamada et al. 1998). In light of findings in the present study, the higher free/total IGF1 ratio as body fat increases would favor protein synthesis (Mauras and Haymond, 2005) and decreased lipolysis favoring fat storage (Leroith and Yakar, 2007; Zizola et al., 2002). This may have potential implications for weight loss. GH is reduced in obesity (Kamoda et al., 2006; Eliakim et al., 2006), which could create a favorable situation for continued weight gain. However, obese children with higher fitness may have less of a GH reduction (Manuscript 1) and actually a lower free/total IGF1 ratio that may be more favorable for weight loss.

Our estimation of VO_2max and body fat percentage is a limitation of the present investigation. However, because collecting reliable VO_2max data on children in a school setting is problematic (Cunningham et al., 1977), especially obese children. Data collection was done in the subject's school some distance from the laboratory, which prevented us from being able to transport the necessary equipment to gather more precise measurements of VO_2max and body composition. However, our methods for predicting VO_2max have a

strong correlation (r=0.80) to measured VO_2max (McMurray et al., 1998) and skinfolds were measured by trained staff only and done in triplicate in accordance with NHANES recommendations (1974).

The major finding of this investigation was that when fitness is accounted for IL-6 levels contribute to alterations in free and total IGF1. Thus, a rational has been developed for further investigation into how fitness and IL-6 may impact the GH-IGF1 alterations. Further our results suggest the free/total IGF1 ratio may be used to further understand alterations of the GH-IGF1 axis across a diverse subject population. The result that free/total IGF1 ratio is positively associated with body fat suggests that large amounts of fat mass increase the free/total IGf1 ratio. The increased free IGF1 in comparison to total IGF1 may play a role in the reduced GH levels often found in obese individuals. Because GH has major effects on metabolism and substrate utilization an increased free/total IGF1 ratio may contribute to continued weight gain in obese children. Future research should expand possible explanations of GH-IGF1 axis alterations to include overweight individuals, activity level of subjects, adipose tissue derived hormones or blood lipids and their relationship to inflammation. In conclusion, these findings support the idea that higher fitness levels may be beneficial for minimizing weight gain in adolescents by minimizing IL-6 levels and possibly preventing alterations of the GH-IGF1 axis seen in obesity.

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CHAPTER SIX

RESEARCH SYNTHESIS

A summary and interpretation of the overall findings on the relationship of the GH-IGF1 axis with obesity and fitness in adolescents are presented below. Before reviewing the significance of the results it is essential to understand that these findings were based on a unique aerobic fitness unit; oxygen uptake per unit of fat free mass. Using the unit VO_2FFM allows for comparison of oxygen utilizing tissue of adolescents with large differences in fat mass. This is important because fat mass has been shown to influence the GH-IFG axis. Much of the research to date used the unit VO_2kg and this measure included both fat free mass and fat mass. Thus, some of the previous findings could be related to fat mass. To determine if analysis using these two different units prodced divergent results both units were used throughout this project. Dispite expected results the majority of analysis revealed similar results for both units indicating, with the present data, that using fitness espressed in units VO_2FFM may not be required for fitness comparison of adolescents with a wide range of weight and fitness status. However, when discrepancies between previous research and present findings are discussed the influence of using VO_2FFM is mentioned.

Major Findings

The results of these investigations have improved the understanding of the GH-IGF1 axis in adolescents and how alterations in obesity and fitness relate to the axis. First, the GH values of the NH group were shown to be significantly higher than the OL group but not OH group. This indicates that obesity is the main determinate for reduced resting GH in children

but that fitness may somewhat mitigate the reduction found in obesity. In contrast to the results found for GH, neither total nor free IGF1were found to be different across the groups, although a trend for free IGF1 to be higher in the OL group compared to the NH group was evident.

In addition to analyzing the effect of obesity and fitness on the GH-IGF1 axis, the relationship of inflammatory markers to the axis was assessed. Interestingly, obesity did not appear to be a major determinate of IL-6. However, those adolescents with elevated fitness had reduced IL-6 levels compared to the low fitness groups regardless of fatness using the unit VO_2FFM which was determined by dividing predicted O_2 uptake by the subject fat free mass instead of the more common total body mass. Further, IL-6 was inversely correlated with fitness (VO_2kg). Based on the differences between the four groups an inverse relationship between IL-6 and VO_2kg was to be expected. When VO_2FFM replaced VO_2kg in the analysis, the relationship still remained. Circulating levels of TNF-α were not different in any of the weight status/fitness groups; but a trend did exist with the NH group having lower levels than the low-fit obese group.

This investigation also examined the influence of inflammatory markers IL-6 and TNF-α on the association between components of the GH-IGF1 axis. Significant relationships were found between GH and total IGF, as well as between free IGF1 and total IGF1. IL-6 had a significant inverse relationship between free IGF1 and total IGF1 when fitness was included in the model, but IL-6 was not associated with the GH and total IGF1 relationship. Since TNF-α was not different by fitness or weight status its relationship to the GH-IGF1 axis was not examined.

Significance of Study and Implication of Results

The most recent estimates indicate that approximately 20% of all U.S. children and adolescent are obese (BMI $\geq 95^{th}$ percentile) highlighting the importance for the health of today's youth (Ogden, 2010). In the transition from overweight to obese several hormonal alterations can occur (Considine et al., 1996; Eliakim et al., 2001). Of particular interest for obesity are the GH-IGF1 axis alterations because of it's involvement in protein synthesis and fatty acid oxidation (Mauras and Haymond, 2005; Leroith and Yadar, 2007; Zizola et al., 2002). Previous research suggests that fitness may impact some of the GH-IGF1 axis alterations, particularly growth hormone and free IGF1 (Eliakim et al., 2006; Ischander et al., 2007). The current research provided data on the resting state of the GH-IGF1 axis in normal weight and obese children and how the axis differs with respect to fitness and fatness.

One of the major findings of the present investigation was that fitness may limit the reduction of GH in obese children and adolescents. Previous research indicates that GH levels are lower in obese subjects compared to normal weight (Attia et al., 1998; Eliakim et al., 2006; Loche et al., 1987). In the present study resting GH values were significantly higher in the NH group compared to the OL group, but not OH group. Significantly lower GH in the OL group indicated that obesity is associated with lower basal GH, but elevated fitness may minimize the effect that obesity has on GH levels. However, these results were concluded from one-way ANOVA run in manuscript one, but to test the independent effect of fitness and fatness an aposteroiri two-way ANOVA showed a significant main effect of fatness with no interaction of fitness and fatness. These results confirm that fatness is the main determining factor for GH which explains the significantly reduced GH in the OL

group. However, fitness may be somewhat responsible for the slight maintenance of GH in the OH group as fitness was the only difference between the OH and OL groups.

While differences in GH were found, neither total nor free IGF1 were different between any of the groups. Therefore, GH levels may not be the sole determinant for total and free IGF1 levels. Taken further, if obese individuals have higher lean body mass with a lower GH but similar total and free IGF levels as normal weight, then obese low-fit individuals may be more sensitive to GH. If an increased sensitivity to GH is occurring one explantion for this may be the elevated insulin levels in obese individual as insulin has been shown to increase GH receptor number and production (Lueng et al., 2000). However, no difference in insulin levels of the OH or OL groups were noted; therefore the slight maintenance of GH in the OH group is not explained by reduced insulin as as originally hypothesized. Taken together, the effect of obesity on GH levels appears greater than the effect of fitness and may not extend to levels of free or total IGF1.

In addition to the reduced GH in the OL group, alterations of IL-6 were found between subjects of the different groups; but not TNF-α. Fitness rather then obesity appeared to influence IL-6 levels, as both highly-fit groups (normal weight and obese) had reduced IL-6 compared to low-fit groups. The majority of IL-6 is released from adipose tissue and is believed to be in response to increased infiltration of macrophages or hypoxia (Eder et al., 2009). The finding that adolescents with higher fitness, not lower levels of adipose, had reduced IL-6 may have implications for the overall role of IL-6. However, the present study did not examine if the reduced IL-6 levels in highly-fit individuals were due to reduction of adipose tissue hypoxia or less macrophage infiltration.

The present investigation demonstrated that IL-6 was inversely correlated with fitness (VO_2kg) which was an expected result. The inverse relationship between IL-6 and VO_2kg was not altered when differences in fat mass were attempted to be accounted for by using VO_2FFM which better focuses on the muscle mass or metabolically active tissue. However, in the present data there was no significant relationship between IL-6 and body fat; thus, accounting for differences in body fat should not have influenced the relationship or using the VO_2FFM does not account for differences in body fat. In contrast to IL-6, TNF-α is minimally affected by fitness or obesity. Therefore, TNF-α may not necessarily need to be included in future investigations, or rather future investigations include both TNF-α and TNF-α receptor activity which may be increased in obesity (Fasshauer et al., 2003) to get a more complete description of potential alterations occuring.

With regard to the influence of IL-6 and TNF-α on the GH-IGF1 axis, the results showed IL-6 had the capacity to impact the relationship between free IGF1 and total IGF1. In this investigation, the relationship between IL-6 and free IGF1was negative, but previous studies found a positive relationship (Fried et al., 1998; Shoelson et al., 2006). When fat mass increases it reduces the unit VO_2kg which significantly reduces the measure of fitness in obese individuals. However, the unit VO_2FFM should be unaffected by fat mass so obese individuals may still have high values for VO_2FFM. Because the influence of fat mass would cause these units to go in opposite directions by defining fitness based on the unit VO_2FFM as was done in this investigation rather then defining fitness groups accourding to VO_2kg may explain the difference in findings. Another possibility is that in the present study 13% of the potential 1486 subjects matched the NH criteria, whereas only 3% matched OH criteria; thus including equal representation from both of these groups may not represent the

norm and may have affected our results. The OH group consisted of all of the subjects in the total sample that met those criteria as compared with the random samples of the other three groups. Yet, because the OH group possessed a high fitness level which resulted in a reduced IL-6 levels whereas the OL had a low fitness level which resulted in elevated IL-6 levels may explain the negative relationship of the present investigation.

Summary of Research Hypotheses

Hypothesis 1a. The OL group will have significantly lower morning fasting GH compared to the NH, NL and OH groups, which will all be similar.

Result: Rejected (only less than OH group not NH and NL groups)

Hypothesis 1b. Morning fasting total IGF1 will not be different between any of the groups.

Result: Accepted

Hypothesis 1c. The morning fasting free IGF1 will be greater in the OL group compared to the OH, NH and NL groups. The OH, NH, and NL groups will not be different from one another .

Result: Rejected (Free IGF1 was not different between any groups)

Hypothesis 2a. Resting IL-6 will be elevated in the OL group compared to the OH group. Resting IL-6 will be similar in NH and NL groups. The NH and NL groups will have similar levels of resting IL-6 production. **Result:** Accepted (OL group elevated compared to OH group); Rejected (NH and NL groups similar)

Hypothesis 2b. Resting IL-6 will be elevated in the OH group compared to the NH and NL groups.

Result: Rejected (IL-6 was reduced in OH compared to NL group and no different from NH group)

Hypothesis 2c. Resting TNF-α will be elevated in the OL group compared to the OH group. Resting TNF-α will be similar in NH and NL groups and the NH and NL groups will have similar resting TNF-α levels.

Result: Rejected (TNF-α was not different between any of the groups)

Hypothesis 2d. Resting TNF-α will be elevated in the OH group compared to the NH and NL groups. **Result:** Rejected (TNF-α was not different between any of the groups)

Hypothesis 3a. In the overall sample, TNF-α will influence the association between GH, total IGF1, and free IGF1. The associations will be modified by obesity and fitness.

Result: Rejected (TNF-α was not correlated with any of these hormones so analysis of TNF-α influence was not carried out)

Hypothesis 3b. In the overall sample, IL-6 will influence the association between GH, total IGF1, free IGF1. The associations will be modified by obesity and fitness.

Result: Patially Accepted (IL-6 influences the fIGF:total IGF1 relationship when fitness is included); Partially Rejected (IL-6 was not correlated with either GH or total IGF1 so analysis to determine if IL-6 influenced the GH:IGF1 relationship was not necessary)

Strengths and Limitations

Much of the literature in this area focuses on differences in obese versus normal weight, ignoring the influence of fitness. However, in the present investigation the effects of fitness were separated from the effects of obesity by using normal weight high-fit, normal weight low-fit, obese high-fit, and obese low fit groups. In addition, differences in fitness were studied as opposed to a response to training (Eliakim et al., 1996; Eliakim 1998; Scheet

2002) which may not be long enough to allow for complete adaptations to occur. Further, in children and adolescents, as opposed to adults, genetics can play a significant role in the fitness level and a training stimulus may not be necessary for a child to be highly fit (Birrer et al., 1987). Thus, the results of the present study more likely represent interactions between components of the GH-IGF1 axis, IL-6, TNF-α, fitness, and obesity that are occurring in a steady-state of fitness and not spurred by sudden increases in energy expenditure (e.g. exercise training). Finally, our measure of fitness was based on the oxygen uptake capability of the FFM (VO_2FFM), not total body mass. Although much of the analysis did not reveal differences between VO_2FFM and VO_2kg the potential differences in the measures had not been made between subjects from a wide range of both fitness and obesity status. Thus, demonstrating that there was no real difference between the two units is noteworthy; however, that lack of difference may be due to the use of several estimations in the equations. Thus these findings should be confirmed using more accurate and precise determinations of oxygen uptake and FFM.

A number of GH-IGF1 axis studies did not measure both total and free IGF1; only total IGF1. One strength of the current investigation was the incorporation of both free and total IGF1. Because free IGF1 represents the bioactive portion of IGF1 including it in any investigation that studies IGF1 and potential influences it can have is important.

The estimations of VO_2max and body fat percentage were limitations of the present investigation. However collecting reliable VO_2max data on children in a school setting is a universal problem (Cunningham et al., 1977); for this reason estimations were used. Data collection was also done in school, some distance from the laboratory, which prevented the transport of the necessary equipment to gather more precise measurements of body fat and

VO_2max. Nonetheless, skinfolds were measured by trained staff only and done in triplicate in accordance with NHANES recommendations (1974) and our methods for predicting VO_2max have a strong correlation (r=0.80) to measured VO_2max (McMurray et al., 1998).

Future Research

Future research in this area should consider examining multiple blood samples throughout the day. GH release follows a pulsatile pattern and has a circadian rhythm (Clasey et al., 2001). Monitoring these patterns throughout the day in normal highly-fit and in obese low-fit individuals would extend the findings of the current investigation to include daily GH release.

Similar to the circadian rhythm, research shows that the exercise response of the GH-IGF1 is blunted in obese individuals (Attia et al., 1998; Eliakim et al., 2006; Loche et al., 1987). Exploring potential differences between GH responses to exercise of highly-fit obese versus low-fit obese individuals would be of interest. Exploring long term changes in fitness and obesity would better answer the question of whether or not obese individual who possess higher fitness have a better chance to lose weight. Future studies should explore the degree to which obesity and fitness influence autocrine/paracrine action of cytokines, specifically TNF-α and TNF-α receptors to determine if they are influencing IL-6 levels. Exploring autocrine/ paracrine action of these cytokines might require tissue and cell culture techniques, thus being rather invasive. However, answering these questions would provide insight into the origin of cytokines in obesity and fitness. Finally, future research in this area should include overweight individuals, as well as normal weight and obese individuals, activity level of subjects, adipose tissue derived hormones, or blood lipids and their relationship to inflammation when controlling for fitness level. Overweight individuals

should be included because their fat mass is not as large as that in obese people and may allow fitness to have a greater influence in the maintenance of GH levels. Activity levels should be included to explain some of the variation in the GH-IGF1 axis and inflammation because activity can influence both (Eliakim et al., 1996; Eliakim et al., 1998). Adipose tissue derived hormones and blood lipids should be included because they are influenced by fitness and obesity (Kasa-Vubu et al., 2006; Maachi et al., 2004) and may be involved in mechanisms explaining GH-IGF1 axis alterations.

Clinical Recommendations

Findings of this investigation suggest that a high aerobic fitness may help reduce some of the GH-IGF1 axis and cytokine alterations seen in obesity, particularly alterations to GH and IL-6. However, based on our results the added adipose tissue resulting in obesity is likely to be the main influence for reduced GH found in obesity. Yet an elevated fitness level appears to ameliorating that reduction to a certain degree. Therefore, in obese subjects weight reduction should be the main goal, but when exercise is included it should focus on improving aerobic fitness, rather then just energy expenditure. If improvements in aerobic fitness are made IL-6 may be reduced and alterations to the GH-IGF1 axis may not be as significant. Therefore, improved fitness leading to reduced IL-6 and a more normal GH-IGF1 axis may over time favor weight loss or at least limit further weight gain. At this point however, these recommendations are speculative and will need continued research support to become conclusive. Our results more fully support that weight reduction should be the primary goal for obese adolescents but also adding a goal of improving fitness may benefital to limit the GH reduction.

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