THE RELATIONSHIPS BETWEEN CHANGES IN WEIGHT STATUS, LEPTIN, CORTISOL, GROWTH HORMONE AND INSULIN RESISTANCE IN YOUTH

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the School of Medicine (Interdisciplinary Program in Human Movement Science).

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ABSTRACT
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The Relationships between Changes in Weight Status, Leptin, Cortisol, Growth Hormone and Insulin Resistance in Youth
(Under the direction of Dr. Robert McMurray)

PURPOSE: The purpose of this investigation was to examine the relationships between changes in weight status that occur over two years and changes in leptin, cortisol, human growth hormone (hGH), and insulin resistance in youth. Additionally, we examined the interrelationships among changes in leptin, cortisol and hGH as well as their relation to changes in insulin. PARTICIPANTS: Data were collected at baseline and two years later for 120 youth purposefully selected from a larger study (CHIC III, Cohort 5, J.S. Harrell, P.I.), to represent four groups: NO (normal weight, >5th and <85th BMI percentile, at baseline and overweight, ≥85th BMI percentile, at follow-up), ON (overweight to normal weight), NN, or OO (normal weight or overweight at both time points, respectively). METHODS: Height and body mass were measured and used to determine BMI percentile. Fasting insulin, leptin, cortisol and hGH were analyzed from plasma via radioimmunoassay. Insulin resistance was estimated via the homeostatic model (HOMA-IR). RESULTS: Two-year changes in BMI percentile were related to: change in HOMA-IR (r=0.26) and changes in leptin (r=0.39), cortisol (r=-0.25) and hGH (r=0.24) (p<0.05). Youth in the NO group had a greater percent change in HOMA-IR (50%) than those in the ON group (-8%) (p<0.05). Additionally, the NO group had greater increases in leptin and greater decreases in cortisol
compared to the ON group (p<0.05); likewise there was an inverse relationship between changes in leptin and cortisol (r=-0.23, p<0.05). No group differences for change in hGH were evident. Changes in leptin were associated with changes in insulin (r=0.29, p<0.05). In the regression model for change in insulin, change in leptin accounted for the greatest amount of variance (R² = 0.09, p<0.05), followed by change in BMI percentile (R² = 0.04, p<0.05). **CONCLUSIONS:** Change in BMI percentile was positively associated with changes in HOMA-IR, leptin and hGH, and negatively related to changes in cortisol. Moving from normal weight to overweight was associated with greater changes in HOMA-IR, leptin and cortisol than moving from overweight to normal weight. These results suggest that natural changes in weight status are related to alterations in several hormones in youth.
ACKNOWLEDGEMENTS

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Finally, I could not have completed this project without the support of my husband, Chris. I am continually amazed by and thankful for his patience, unselfishness and unwavering devotion. I would also like to thank my family, friends and fellow graduate students for their encouragement and support throughout this journey.
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CHAPTER ONE
Introduction

Background

Insulin resistance (IR) is a precursor of type II diabetes and develops during pre-diabetes (ADA, 2005). Normally, the release of insulin and uptake of glucose are coupled. However, as insulin resistance develops fat, muscle and liver cells become resistant to insulin’s actions (Garvey and Birnbaum, 1993), and require greater amounts of insulin to maintain normal blood glucose levels. Eventually, the increased insulin levels are not sufficient to lower blood glucose and hyperglycemia develops; this condition is known as pre-diabetes. As IR progresses, greater amounts of insulin are produced until the pancreas can no longer keep up with the body’s insulin requirements. Once this happens, insulin production declines and type II diabetes develops (ADA, 2005; Garvey and Birnbaum, 1993).

One factor associated with the development of IR in youth is being overweight. The prevalence of overweight in youth has more than doubled since the late 1970’s and early 1980’s (Ogden et al., 2002). The most recent estimates show that 37% of children ages 6 to 11 are at risk for or are overweight (defined as BMI ≥ 85th percentile) (Ogden et al., 2007). Among overweight and obese youth, the prevalence of IR is 52 to 72% (Druet et al., 2006; Lee, Okumura, et al., 2006). Similarly, obese children are ~13 times more likely to have elevations in fasting insulin, a marker of IR, compared to normal weight children (AOA website, 2007). The alarming prevalence of obesity and IR and their strong relationship have
emerged as significant health issues for youth (ADA, 2000). Therefore understanding the factors that influence the relationship between obesity and IR is critical.

Researchers have identified numerous factors that affect the relationship between overweight status and insulin resistance in youth. They include level of physical fitness (Ahn et al., 2004; Allen et al., 2007; Ball et al., 2004; Nassis et al., 2005), cytokines such as TNF-α, IL-6, adiponectin or resistin, and hormones such as leptin, cortisol and human growth hormone (hGH) (Cruz et al., 2005; Rosenberg et al., 2005; Wisse, 2004). The current investigation focused on the hormonal factors and their relationship with insulin and IR in youth.

Much of what is known regarding the progression of IR in youth has come from longitudinal studies of adolescents involved in weight change interventions. These studies have shown that weight status changes are associated with alterations in resting hormone levels. For example, when normal weight individuals become overweight, leptin and cortisol levels increase, while hGH declines (Reinehr and Andler, 2004a; Savoye et al., 2002). Conversely, when overweight individuals become normal weight, leptin and cortisol decrease and hGH increases (Argente et al., 1997; Falorni et al., 1997; Gallistl et al., 2001; Geldszus et al., 1996; Lazzer, Vermorel, et al., 2005; Pilcova et al., 2003; Reiterer et al., 1999; Reinehr et al., 2005; Sudi et al., 2001). Thus, research shows that changes in weight status are related to hormonal alterations in youth.

In addition to the associations between changes in weight status and leptin, cortisol and hGH, the hormones and their interrelationships also influence IR. For example, leptin has been shown to inhibit the release of cortisol, hGH and insulin (Bornstein et al., 1997; Carro et al., 1997; Cavagnini et al., 2000; Coutant et al., 1998; Fehmann et al., 1997; Havel,
2004; Kieffer and Habener, 2000; Leal-Cerro et al., 2001; Pralong et al., 1998; Seufert et al., 1999; Seufert, 2004; Wauters et al., 2000). Research also shows that cortisol inhibits hGH and insulin release (Barbarino et al., 1990; Delaunay et al., 1997; Ghizzoni et al., 1996; Lambillotte et al., 1997; Ling et al., 1998), while stimulating leptin release (Askari et al., 2000; Berneis et al., 1996; Ghizzoni and Mastorakos, 2003; Leal-Cerro et al., 2001; Nishiyama et al., 2000; Vettor et al., 2005; Wabitsch et al., 1996; Wauters et al., 2000). Interestingly, insulin has been shown to stimulate the release of leptin (Barr et al., 1997; Boden et al., 1997; Havel, 2000; Havel, 2004; Kieffer and Habener, 2000; Lee et al., 2001; Wabitsch et al., 1996; Wauters et al., 2000), while inhibiting the release of cortisol (Jamieson et al., 1995). However, some studies have produced conflicting information regarding some interrelationships and their relation to IR. For example, hGH has been shown to stimulate, inhibit or have no effect on the release of leptin (Berneis et al., 1996; Cavagnini et al., 2000; Coutant et al., 1998; Fain and Bahouth, 2000; Ghizzoni et al., 2001; Isozaki et al., 1999; Lee et al., 2001; Wauters et al., 2000), while it’s influence on the release of cortisol is not well understood. Additionally, if levels of these hormones change in response to weight status changes, the interrelationships among these hormones and their influence on IR may be modified as well.

To our knowledge, no previous investigation has examined the interplay of these hormones and their relation to IR following natural changes in weight status that are not a result of an intervention, in children and adolescents. Additionally, the examination of four weight status categories is unique as no previous studies have examined these relationships in participants undergoing weight status increases, decreases, or maintenance.
Therefore, the purposes of this investigation were: 1) to determine the relationship between changes in weight status and insulin resistance, 2) to determine the association between changes in weight status and changes in resting levels of leptin, cortisol, and growth hormone, 3) to quantify the strength of interrelationships among leptin, cortisol and hGH, as well as their relation to change in insulin and, 4) to determine the relationship of changes in these hormones and weight status with changes in insulin in youth.

**Operational Definitions**

**Insulin Resistance (IR)** is defined using the homeostatic model assessment (HOMA-IR).

HOMA-IR is calculated using the following equation: 
\[
\text{HOMA-IR} = \frac{\text{fasting insulin concentration (µU/mL)} \times \text{fasting glucose concentration (mmol/L)}}{22.5}
\]  
(Matthews et al., 1985). A larger HOMA-IR indicates greater insulin resistance.

**Insulin Sensitivity** is defined as the inverse of insulin resistance.

**Time 1 (t₁)** is defined as the first measurement point.

**Time 2 (t₂)** is the second measurement point, taken two years after time 1.

**Overweight** is defined as a BMI ≥ 85th percentile for the child’s age and sex.

**Normal weight** is defined as a BMI >5th and <85th percentile for the child’s age and sex.
**Change in Weight Status** is defined as a classification of normal weight at t₁ and overweight and t₂, or vice versa.

**Maintenance of Weight Status** is defined as a classification of normal weight at t₁ and t₂, or overweight at t₁ and t₂.

**NO group** is defined as participants who were normal weight at t₁ and overweight at t₂.

**NN group** is defined as participants who were normal weight at t₁ and t₂.

**ON group** is defined as participants who were overweight at t₁ and normal weight at t₂.

**OO group** is defined as participants who were overweight at t₁ and t₂.

**VO₂/kgFFM** is defined as maximal aerobic power, expressed in mL of oxygen per kilogram of fat free mass per minute.

**Research Aims and Hypotheses**

The purposes of this investigation were addressed by four research aims. Figure 1 depicts the research design and how the relationships between the variables are assessed in each research aim. The four aims and accompanying hypotheses are described below the figure.
The first research aim evaluated the relationship among change in weight status and change in IR, the primary outcome variable of this investigation, and this aim is addressed in Manuscript #1. The second research aim assessed the relationship between change in weight status and changes in leptin, cortisol and hGH, while the third aim identified the interrelationships between changes in these hormones. Both of these aims are covered in Manuscript #2. Finally, Manuscript #3 describes the relationship between changes in these hormones and changes in insulin, along with the fourth aim which determined the strength of the relationship between each of the following variables and insulin change: changes in weight status, leptin, cortisol and hGH.
Aim #1. To determine the relationship between change in weight status and change in insulin resistance.

(Hypotheses 1a and 1b analyze weight as a categorical variable = weight status; Hypothesis 1c analyzes weight as a continuous variable = BMI percentile. The dependent variable for all hypotheses is the continuous variable, HOMA-IR.)

**Hypothesis 1a.** Insulin resistance will increase more in participants who were normal weight at t₁ and overweight at t₂ (NO) than in participants who were normal weight at both time points (NN).

**Hypothesis 1b.** Insulin resistance will decrease more in participants who were overweight at t₁ and normal weight at t₂ (ON) than in participants who were overweight at both time points (OO).

**Hypothesis 1c.** Change (t₂-t₁) in BMI percentile will be positively related to change in insulin resistance.
Aim #2. To determine the relationship among change in weight status and changes in leptin, cortisol and human growth hormone.

(Hypotheses 2a and 2b analyze weight as a categorical variable = weight status; Hypothesis 2c analyzes weight as a continuous variable = BMI percentile.)

Hypothesis 2a. Participants who were normal weight at t1 and overweight at t2 (NO) will have greater increases in leptin and cortisol and greater decreases in hGH compared to participants who were normal weight at both time points (NN).

Hypothesis 2b. Participants who were overweight at t1 and normal weight at t2 (ON) will have greater decreases in leptin and cortisol and greater increases in hGH compared to participants who were overweight at both time points (OO).

Hypothesis 2c. Change in BMI percentile will be positively related to change in leptin and cortisol and negatively related to change in hGH.

Aim #3. To determine the relationship between changes in leptin, cortisol and hGH and to determine the relationship of each of these hormones with changes in insulin.

Hypothesis 3a. Change in leptin and cortisol will be positively correlated to each other and negatively correlated to change in hGH.

Hypothesis 3b. Change in insulin will be positively correlated with changes in leptin and cortisol and negatively correlated to change in hGH.

Aim #4. To determine how much of the variance in change in insulin is attributable to changes in BMI percentile, leptin, cortisol and hGH.

(Hypotheses 4a-4e analyze weight as a continuous variable = BMI percentile.)
**Hypothesis 4a.** Change in BMI percentile will explain a significant portion of the variance in change in insulin.

**Hypothesis 4b.** Change in leptin will explain a significant portion of the variance in change in insulin.

**Hypothesis 4c.** Change in cortisol will explain a significant portion of the variance in change in insulin.

**Hypothesis 4d.** Change in hGH will explain a significant portion of the variance in change in insulin.

**Hypothesis 4e.** Change in leptin will account for the greatest amount of variance in change in insulin.
CHAPTER TWO

Manuscript One

The Relationship between Change in Weight Status and Change in Insulin Resistance in Youth

Kristin S. Ondrak, Robert G. McMurray, Claudio L. Battaglini, Kelly R. Evenson, Joanne S. Harrell
ABSTRACT

INTRODUCTION: Few studies have investigated the relationship between change in weight status and change in insulin resistance (IR) in youth. The purpose of this study was to examine the change in IR in youth who moved from a normal weight to overweight status and youth who were overweight and became normal weight over a two year period.

METHODS: Data were collected at baseline and two years later for 120 youth (9.8 ± 1.0 years at baseline). Participants were divided into four groups based upon their weight status at baseline and follow-up: NN (normal – normal), NO (normal to overweight), ON (overweight to normal), and OO (overweight – overweight). Normal weight was defined as > 5th and < 85th BMI percentile, while overweight was ≥ 85th body mass index (BMI) percentile for age and sex. Insulin and glucose were measured at both time points and were used to quantify insulin resistance via homeostatic model assessment equation (HOMA-IR). T-tests were used to compare the percent change in HOMA-IR between the groups and Spearman correlations were calculated to assess the relationship between change in BMI percentile and change in HOMA-IR.

RESULTS: Youth in the NO group had a greater percent change in HOMA-IR (50%) than those in the NN (2%) or ON groups (-8%). There was no difference in percent change in HOMA-IR between the OO and ON groups, however. The association between change in BMI percentile and change in HOMA-IR was moderate in strength (r=0.41) when only the NO and ON groups were considered, and weak, but still significant when all participants were analyzed (r=0.26).

CONCLUSION: Moving from normal to overweight status was associated with large increases in HOMA-IR in youth. Supported by NINR #NR01-1837.
INTRODUCTION

Insulin resistance (IR) is a precursor to type II diabetes and recent reports show that 52 to 72% of overweight and obese youth have IR (Druet et al., 2006; Lee, Okumura, et al., 2006). While numerous cross-sectional studies have noted positive associations ($r=0.31-0.85$) between insulin and measures of adiposity in youth (Bacha et al., 2006; Gower et al., 1999; Lee, Okumura, et al., 2006; Roemmich et al., 2002; Thorsdottier et al., 2006; Travers et al., 1995; Young-Hyman et al., 2001), the relationships between change in weight status and change in IR have not been examined thoroughly. The few longitudinal studies conducted in this area in youth have consistently reported that change in weight is positively associated with change in insulin or IR, and negatively associated with insulin sensitivity (Johnson et al., 2001; Lazzer, Vermorel, et al., 2005; Reinehr et al., 2004).

The current investigation will add to our understanding of the relationship between overweight and IR by directly comparing the percent change in IR in youth who were normal weight at baseline and overweight two years later, to those who were overweight and became normal weight. Additionally, comparisons will be made with participants who remained normal weight or overweight over the two year study period. We hypothesized that a larger increase in IR would occur in the participants who became overweight compared to those who became normal weight or those who remained normal weight. Similarly, we expected to find a larger decline in IR in participants who were overweight and became normal weight, compared to those who remained overweight two years later. Change in weight status was also analyzed using the continuous variable of body mass index (BMI) percentile, since this variable adds unique information by indicating the magnitude of change. We hypothesized
that change in BMI percentile over the two year study period would be positively related to changes in IR.

METHODS

Participants

The participants were enrolled in the Cardiovascular Health in Children III (CHIC) study, Cohort 5 (J.S. Harrell, P.I.). The CHIC III study was a longitudinal investigation assessing risk factors for cardiovascular disease in youth from rural North Carolina, taking place from 2000 to 2003. A total of 120 children and adolescents were selected from the 1566 participants belonging to Cohort 5, based on their weight status, as described in the Weight Status Groups section. Of the original 1566 participants, the mean ages at baseline and follow-up were 9.6±1.1 years and 11.5±1.0 years, respectively. The sex and racial composition of this cohort was: 50% female and 50% male, 53% Black, 36% White, and 11% Other.

Procedures

All data were collected at baseline and two years later, termed baseline and follow-up in this study. The participants and their parents signed assent and consent forms, respectively, prior to data collection and all procedures were approved by the Institutional Review Board at the University of North Carolina at Chapel Hill. Data were collected at the participants’ schools by trained research assistants. Height and body mass were measured using a stadiometer (Perspective Enterprises, Portage, MI) and an electronic scale (Model 2101KL, Healthometer Medical, Bridgewater, IL), respectively. BMI was calculated from these measurements (kg/m²).
Researchers have noted that pubertal status (Cook et al., 1993; Guzzaloni et al., 2002; Roemmich et al., 2002; Travers et al., 1995) and cardiovascular fitness (Allen et al., 2007) influence HOMA-IR in youth, and as a result, both measures were included in this investigation. Pubertal development was quantified using self-report questionnaires (Petersen et al., 1988). Cardiovascular fitness was assessed using a multi-stage submaximal test, the Physical Work Capacity 195 (PWC\textsubscript{195}), completed on a cycle ergometer (McMurray et al., 1998). The workload corresponding to a heart rate of 150-170 was used to predict the maximal volume of oxygen consumed (VO\textsubscript{2}) and strong correlations ($r=0.807$) have been shown between this test and measured VO\textsubscript{2}max in children (McMurray et al., 1998). The results of the test were expressed in ml of oxygen per kg fat free mass per min (VO\textsubscript{2}/kg\textsubscript{FFM}), to eliminate the effect of fat mass. Fat free mass was estimated from measures of percent body fat, obtained via sum of skinfolds taken at the subscapular and triceps sites. These measures were taken in triplicate using Lange calipers (Cambridge Scientific, Cambridge, MD), according to the guidelines set forth by NHANES III (1974). Equations specific to each sex, race and pubertal status were then applied to estimate percent body fat (Slaughter et al., 1988). In order to ensure strong intra and inter-rater reliability, respectively, all research assistants were trained by the same investigator and height, body mass and skinfolds were measured by more than one RA for every tenth participant. If the measurement was greater than preestablished quality control criteria, the research assistant was not permitted to participate in future measure.

Blood draws were performed using standard venipuncture methods, between 7 and 9 am, following a 12 hour fast. The samples were centrifuged and the plasma was stored in vials and frozen in a -80°C freezer for later analysis. Insulin was measured from the stored
plasma using radioimmunoassay procedures (Linco, St. Charles, MO), while glucose was
analyzed via automated hexokinase oxidase procedures. These values were used to calculate
insulin resistance (IR) using the homeostatic model assessment equation (HOMA-IR); fasting
insulin concentration (µU/mL) * fasting glucose concentration (mmol/L) / 22.5 (Matthews et
al., 1985).

**Weight Status Groups**

Weight status was analyzed using both continuous and categorical variables. The
continuous variable of BMI percentile was derived from the Centers for Disease Control and
Prevention (CDC) growth charts from the year 2000 (CDC, 2000). To create the categorical
weight status variable, participants were selected from the larger cohort and divided into four
groups (NO, NN, ON, OO) based upon their BMI percentile at baseline and follow-up.
Normal weight was defined as >5\textsuperscript{th} and <85\textsuperscript{th} BMI percentile for age and sex, while
overweight was defined as ≥ 85\textsuperscript{th} BMI percentile for age and sex. The NO group was
comprised of 35 participants who were normal weight at baseline and overweight at follow-
up. These participants were randomly selected from participants in the original cohort who
met these criteria and had complete data. A comparison group, NN, was comprised of
participants who were normal weight at both time points. This group was formed by
identifying participants from the original cohort who matched those in the NO group by sex,
race (black, white, or other) and pubertal status at baseline. From the eligible participants,
we randomly selected 35 participants for the NN group. Conversely, the ON group was
comprised of 25 participants who were randomly selected from all those who were
overweight at baseline and normal weight at follow-up, with complete data. The
 corresponding comparison group, OO, was comprised of 25 participants who were
overweight at both time points. These participants were randomly selected from eligible participants who matched those in the ON groups by sex, race and pubertal status at baseline.

**Statistical Analysis**

A one-way ANOVA was computed to compare the following variables between the four weight status groups at baseline: age, pubertal stage, body mass, height, BMI, BMI percentile, VO$_2$/kg$_{FFM}$, glucose, insulin, and HOMA-IR. A Mantel-Haenszel chi-square was calculated to assess group differences at baseline for the categorical variables race and sex.

The percent change in HOMA-IR ([(follow-up – baseline) / baseline]) was calculated for each participant. To assess group differences in percent change in HOMA-IR, t-tests were computed between the NO and ON groups, NN and NO groups, and the OO and ON groups. A Bonferroni correction was applied to account for multiple t-tests, yielding an alpha of 0.017 for these analyses.

The relationship between percent change in IR and change in BMI percentile was examined using Spearman correlations. To further explore the relationship between these variables, we also calculated Spearman correlations using only those participants in the NO and ON groups, since they changed their weight status over the two year study period. Partial correlations were calculated between change in BMI percentile and percent change in HOMA-IR, after controlling for changes in pubertal status and VO$_2$/kg$_{FFM}$. Finally, to determine which variable was driving the change in HOMA-IR, we repeated all analyses using changes in insulin and glucose as the dependent variables. The alpha level was set at p<0.05 throughout the investigation, unless noted otherwise. In addition, 95% confidence intervals were computed for the correlations. All statistical analyses were conducted using SAS Statistical Software, Version 9.1 (Cary, NC).
RESULTS

Participant Characteristics and Group Differences at Baseline

The characteristics of the four groups at baseline and the two-year follow-up measurement (mean ± standard deviation) are shown in Table 1. At both time points, there were 17 females and 18 males in the NN and NO groups, and 14 females and 11 males in OO and ON groups. There were no group differences for sex, but there were significant differences for race, such that the OO and ON groups had fewer Black participants than the NN and NO groups (p<0.05). By design, the mean BMI percentile of the OO and ON groups were greater than the NN and NO groups at baseline (p<0.05). Participants in the NO were approximately one year younger than participants in the other groups (p<0.05), despite similar medians and ranges of pubertal stages in all four groups (p>0.05). There were no group differences in glucose at baseline, but the OO group had larger mean values for insulin and HOMA-IR, compared to all other groups (p<0.05).

Regardless of weight status group, the mean HOMA-IR values of all normal weight participants were below cut-points for IR, and therefore not insulin resistant (Lee, Okumura et al., 2006). However, the mean HOMA-IR values for overweight participants were greater than some of these cut-points and could be considered insulin resistant. As expected, the mean values for the NO and ON groups show a trend of insulin resistance only when the participants were overweight. There were numerous participants with elevated insulin values (>20 µU/mL); 16 at baseline and 21 at follow-up. Similarly, 16 participants had clinically elevated blood glucose levels (≥ 100 mg/dL) at baseline and follow-up. This suggests that some participants were developing insulin resistance and impaired fasting glucose levels.
Changes in HOMA-IR

The NO group had a greater percent change in HOMA-IR compared to the ON group, as shown in Table 2 (49.7% vs.-7.7%, respectively, p=0.006). To further investigate the influence of weight status change on IR, we compared the percent change in HOMA-IR between the NO and NN groups and between the OO and ON groups. The percent change in HOMA-IR was much greater in the NO group than the NN group (49.7% vs. 2.1%, p=0.009). However, no differences were found between the percent change in HOMA-IR in the OO and ON groups (p=0.626).

The Spearman correlation between change in BMI percentile and percent change in HOMA-IR was 0.26 (p=0.004) when all participants were included, however the strength of this relationship increased to 0.41 (p=0.001) when only the NO and ON groups were analyzed (Table 3). Controlling for changes in fitness (VO_2/kg_FFM) and pubertal status did not affect the relationship between change in BMI percentile and percent change in HOMA-IR, as the partial correlations were nearly identical to the original correlations (Table 3).

Changes in Insulin and Glucose

To establish which variable had the greatest influence on HOMA-IR, we repeated all analyses using glucose and insulin in place of HOMA-IR. The group differences for percent change in insulin were identical to those for percent change in HOMA-IR (Table 2). Likewise, the Spearman correlations and partial correlations for changes in BMI percentile and percent change in insulin were also extremely similar to those for percent change in HOMA-IR (Table 3).

When analyzing percent change in glucose, there were no significant differences between any of the following groups: NO and ON (p=0.10), NN and NO (p=0.36), or OO
and ON (p=0.68). Similarly, the correlations between percent change in glucose and change in BMI percentile, were not significant when all participants were used (p=0.12), or when only the NO and ON groups were examined (p=0.12). As a result, no further analyses with glucose were conducted.
Table 1. Descriptive Statistics (mean ± standard deviation) at baseline and follow-up two years later, presented by weight status group.

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<tr>
<th></th>
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<th>ON (n=25)</th>
<th>OO (n=25)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
<td>Follow-up</td>
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<tr>
<td>Sex (females, males)</td>
<td>17, 18</td>
<td>17, 18</td>
<td>14, 11</td>
<td>14, 11</td>
</tr>
<tr>
<td>Race (black, white, other)</td>
<td>21, 13, 1</td>
<td>21, 13, 1</td>
<td>8, 15, 2</td>
<td>8, 15, 2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.8±0.9</td>
<td>11.7±0.9</td>
<td>9.2±1.0</td>
<td>11.1±1.0</td>
</tr>
<tr>
<td>Median Pubertal Stage</td>
<td>2 (1-4)</td>
<td>3 (1-5)</td>
<td>2 (1-4)</td>
<td>3 (1-4)</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>34.2±5.3</td>
<td>42.8±6.5</td>
<td>34.9±5.6</td>
<td>49.8±7.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>141.0±7.3</td>
<td>152.4±8.1</td>
<td>137.0±8.2</td>
<td>148.4±8.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.1±1.6</td>
<td>18.4±1.7</td>
<td>18.5±1.1</td>
<td>22.5±1.8</td>
</tr>
<tr>
<td>BMI Percentile</td>
<td>50.8±24.4</td>
<td>51.8±23.1</td>
<td>76.0±8.0</td>
<td>90.2±3.1</td>
</tr>
<tr>
<td>VO₂/kgFFM</td>
<td>51.5±10.9</td>
<td>53.8±9.7</td>
<td>47.9±10.4</td>
<td>47.9±10.0</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>88.8±9.7</td>
<td>92.4±7.7</td>
<td>90.6±8.1</td>
<td>92.4±9.7</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>12.1±10.3</td>
<td>10.8±8.2</td>
<td>13.3±7.1</td>
<td>16.7±11.3</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.7±2.4</td>
<td>2.5±2.1</td>
<td>3.0±1.8</td>
<td>3.9±3.0</td>
</tr>
</tbody>
</table>

*p<0.05, NO < all other groups at baseline
†p<0.05, NN and NO < ON < OO at baseline
‡p<0.05, NN < NO < ON and OO at baseline
^p<0.05, NN < OO at baseline
**p<0.05, NN, NO and ON < OO at baseline

Table 2. Percent change in glucose, insulin, and HOMA-IR (mean ± standard deviation), by weight status group.

<table>
<thead>
<tr>
<th></th>
<th>NN</th>
<th>NO</th>
<th>ON</th>
<th>OO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent ∆ in HOMA-IR</td>
<td>2.1±48.5</td>
<td>49.7±93.5</td>
<td>-7.7±43.0</td>
<td>-0.8±55.5</td>
</tr>
<tr>
<td>Percent ∆ in Insulin</td>
<td>-2.8±42.8</td>
<td>43.8±84.8</td>
<td>-7.2±38.2</td>
<td>1.6±52.2</td>
</tr>
<tr>
<td>Percent ∆ in Glucose</td>
<td>5.1±12.9</td>
<td>2.4±11.2</td>
<td>-2.3±10.0</td>
<td>-3.6±12.3</td>
</tr>
</tbody>
</table>

*p<0.05, NO vs. NN and ON
Table 3. Spearman correlations (95% confidence intervals) between changes in BMI percentile and percent change in HOMA-IR and insulin, before and after controlling for changes in pubertal status and VO$_2$/kgFFM.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta$ BMI percentile</th>
<th>$\Delta$ BMI percentile, controlling for changes in pubertal status and VO$_2$/kgFFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent $\Delta$ HOMA-IR (all participants)</td>
<td>0.26 (0.08 to 0.42)</td>
<td>0.25 (0.07 to 0.41)</td>
</tr>
<tr>
<td>Percent $\Delta$ HOMA-IR (NO and ON only)</td>
<td>0.41 (0.17 to 0.60)</td>
<td>0.42 (0.17 to 0.61)</td>
</tr>
<tr>
<td>Percent $\Delta$ insulin (all participants)</td>
<td>0.25 (0.08 to 0.41)</td>
<td>0.25 (0.07 to 0.41)</td>
</tr>
<tr>
<td>Percent $\Delta$ insulin (NO and ON only)</td>
<td>0.42 (0.17 to 0.61)</td>
<td>0.42 (0.17 to 0.60)</td>
</tr>
</tbody>
</table>

All correlations were $p<0.05$

DISCUSSION

Main Findings

This investigation is one of the first to explore the relationship between changes in weight status and changes in HOMA-IR in youth. We found that the percent change in HOMA-IR was greater in participants who became overweight than in participants who became normal weight. There were also significant, but weak correlations between change in BMI percentile and percent change in HOMA-IR. These correlations were not affected by changes in pubertal status or fitness (VO$_2$/kgFFM), suggesting that they may not influence this relationship.

Changes in HOMA-IR

As expected, HOMA-IR increased in the NO group and decreased in the ON group. However, a key finding was that the change in HOMA-IR was much greater for participants who became overweight (50%), compared to participants who were overweight and became normal weight (-8%). These data suggest that efforts to prevent normal weight youth from becoming overweight are necessary. The change in BMI percentile over the two year period
was remarkably similar in these groups, although it increased in the NO group (14.2 units) and decreased in the ON group (13.9 units). Additionally, the HOMA-IR for the NO group at baseline was identical to the HOMA-IR for the ON group at follow-up (Table 1). However, the HOMA-IR in the ON group at baseline was lower than expected, which may explain the relatively small percent reduction in HOMA-IR in the ON group.

When comparing participants who became overweight to those that remained normal weight, we saw that the 50% increase in HOMA-IR in the NO group was much larger than the 2% increase seen in the NN group. This demonstrates that becoming overweight is related to substantial increases in HOMA-IR that are not present when normal weight status is maintained. To our knowledge, no previous investigation has directly compared participants using these weight categories.

The lack of differences in the percent change in HOMA-IR between the OO and ON groups may be attributable to several factors. First, the mean HOMA-IR value for the ON group was 3.3 units at baseline, which was slightly lower than expected for overweight participants. This makes it more difficult to observe large changes in response to weight status change. Additionally, research has shown that insulin sensitivity declines as youth mature and pass through puberty (Cook et al., 1993; Guzzaloni et al., 2002; Travers et al., 1995). In this investigation, most participants advanced by at least one pubertal stage over the two year period. The ON group had the highest median pubertal stage at follow-up, which may have increased their resistance to insulin and decreased any weight-related reductions in IR. Another factor that may explain the similar changes in HOMA-IR between the OO and ON groups is the magnitude of weight status change. Previous studies have reported that the change in insulin sensitivity is related to the amount of change in BMI.
For example, significant improvements in insulin sensitivity were only found when obese children decreased their BMI Standard Deviation score by $\geq 0.5$ over a one year period, which was equal to a reduction in BMI of 2.2 in this group (Reinehr, Kiess et al., 2004). A similar investigation found that reductions in BMI SD score of $\geq 0.5$ were also needed in order to elicit significant reductions in HOMA-IR after one year, and the reduction of BMI in this group was 3.2 (Reinehr and Andler, 2004b). In the current investigation, the ON group had a mean BMI reduction of 0.9 units, which suggests that the weight status changes may have been too small in magnitude to influence IR. Finally, the degree of overweight may influence the amount of change in IR. For example, Savoye et al. (2007) found reductions in HOMA-IR of 1.5 units in a group of obese participants who decreased their BMI by 1.7 units in one year. The starting BMI for their group was 35.8 $\text{kg/m}^2$, however, which is much higher than the BMI for the ON group in our investigation. Taken together, these factors may explain why we saw a small percent decrease in IR in the ON group despite a 14 point reduction in BMI percentile and subsequently, why we did not observe significant differences between the OO and ON groups.

**Weight Status as a Continuous Variable**

When change in weight status was analyzed using the continuous variable of BMI percentile change, stronger relationships emerged. The Spearman correlation between change in BMI percentile and percent change in HOMA-IR in the NO and ON participants was moderate in strength ($r=0.41$). The partial correlations between change in BMI percentile and percent change in HOMA-IR were nearly identical after controlling for changes in pubertal status and $\text{VO}_2$ per kg of fat free mass, suggesting that these variables did
not influence these relationships. When these correlations were repeated using all participants, the relationships were weaker but the trends were the same (Table 3). These results disagree with cross-sectional studies that show positive relationships between pubertal status (Roemmich et al., 2002) and IR and negative relationships between fitness and IR in children and adolescents (Allen et al., 2007). However, the changes in pubertal status and fitness in our investigation were generally quite small which may explain their insignificant role in these relationships. The majority of participants (55%) increased by one pubertal stage over the two year study period, while 28% did not change pubertal stage, 14% increased by two stages and only 3 participants advanced three stages. Similarly, changes in fitness over the two year period were small and ranged from -0.08 to 2.80 ml/kgFFM/min.

**HOMA-IR vs. Fasting Insulin**

In general, the results were nearly identical when either percent change in HOMA-IR or percent change in insulin was used as the dependent variable. This suggests that insulin was the driving force behind HOMA-IR, and the influence of glucose was quite small. This was also evident in the group comparisons at baseline, as there were no group differences in glucose, but the OO group had significantly higher insulin and HOMA-IR compared to all other groups. Our findings agree with previous research showing strong associations between fasting insulin and HOMA-IR (Wilson et al., 2004). In fact, the correlation between HOMA-IR and insulin was 0.98 at baseline and 0.99 at follow-up (p<0.05). Thus, HOMA-IR did not yield stronger associations than insulin alone, and this agrees with previous research supporting the use of HOMA-IR (Keskin et al., 2005; McAuley et al., 2007).

**Strengths and Limitations**
As with any investigation, there are inherent strengths and limitations. One strength is the inclusion of participants who changed their weight status favorably (ON group) and unfavorably (NO), and the creation of comparison groups (NN and OO) matched to the weight status change groups by sex, race, and pubertal status at baseline. This study is one of the few to describe HOMA-IR in youth who underwent favorable changes in weight status by moving from overweight to normal weight, as the majority of research focuses on youth who move from normal weight to overweight status. Additionally, the use of BMI percentiles allows for easy comparison between youth and their peers, since BMI percentiles take into account the increase in both height and weight that occur during growth and development. A final strongpoint of this investigation is the consideration of pubertal status and fitness, as it is likely that they are related to weight status and HOMA-IR in youth.

Some limitations of this investigation include the relatively small sample sizes in each group, and the breadth of BMI percentiles in the overweight category (≥ 85th percentile). If more participants were included, the overweight groups may have been divided further, into the “at risk for overweight” and “overweight” groups, as described by the CDC (CDC, 2000). Also, it is important to note that there were only 25 participants each in the ON and OO groups, as opposed to the 35 participants in the NN and NO groups. However, despite the small sample size, we had sufficient statistical power at the 0.80 level, to detect group differences. Additionally, several cut-points in HOMA-IR have been used to classify IR in children and adolescents, ranging from 3.16 to 4.0, yet there is no universally accepted value for use in youth (Keskin et al., 2005; Lee, Okumura, et al., 2006). This complicates the interpretation of results, as different conclusions can be drawn depending upon which cut-point is used. As a result, we did not dichotomize individuals as insulin resistant or not.
Once a consensus is reached regarding these cut-points, normal and overweight participants could be compared using these insulin resistance classifications. Finally, we are unable to determine temporality of these relationships using the current design. As a result, we cannot determine if changes in weight status caused changes in HOMA-IR, or vice versa, and instead can only comment upon their associations.

**Summary**

Participants who became overweight over the two year study period had substantial increases in HOMA-IR and these changes were greater than those who were overweight and became normal weight. These results suggest that the prevention of overweight in youth is of major importance.
REFERENCES


CHAPTER THREE
Manuscript Two

Changes in Leptin, Cortisol and Human Growth Hormone: Their Interrelationships and Their Relation to Changes in Weight Status in Youth

Kristin S. Ondrak, Robert G. McMurray, Anthony C. Hackney, Joanne S. Harrell
ABSTRACT

INTRODUCTION: The relationship between changes in weight status and changes in leptin, cortisol and human growth hormone (hGH) are not well understood in youth. Similarly, no previous studies have described the interrelationships among changes in leptin, cortisol and hGH in youth undergoing changes in weight status. METHODS: This longitudinal study examined 120 youth at baseline (t₁) and two years later (t₂). The participants were selected from a larger cohort according to their BMI percentile at t₁ and t₂ and divided into four weight status groups: NN (normal weight at t₁ and t₂), NO (normal weight at t₁ and overweight at t₂), ON (overweight at t₁ and normal weight at t₂), OO (overweight at t₁ and t₂). Normal weight was defined as > 5th and < 85th BMI percentile, and overweight was ≥ 85th BMI percentile for age and sex. Blood draws were taken at t₁ and t₂ and leptin, cortisol and hGH were measured from the plasma via radioimmunoassay procedures. ANCOVAs were conducted to assess group differences in changes in leptin, cortisol and hGH, with race, sex, and hormone values at t₁ as covariates. Partial Pearson correlations were conducted to determine the relationship between change in BMI percentile and changes in the leptin, cortisol and hGH, after controlling for race and sex. The interrelationships among changes in these hormones were also assessed using partial Pearson correlations, controlling for race and sex. RESULTS: The NO group had greater increases in leptin and greater decreases in cortisol compared to the ON group (p<0.05). There were no group differences for change in hGH, however. There were significant correlations among change in BMI percentile and changes in leptin (r=0.39), cortisol (r=-0.25) and hGH (r=0.24). Finally, there was an inverse relationship between change in leptin and change in cortisol (r=-0.23, p<0.05). CONCLUSION: These results suggest that changes in leptin, cortisol and hGH were more
strongly related to changes in BMI percentile than with changes in one other. Supported by NINR #NR01-1837.
INTRODUCTION

Cross-sectional research shows that resting levels of several metabolic hormones are affected by weight status in youth. For example, obese youth have higher levels of leptin and cortisol (Ahmed et al., 1999; Aygun et al., 2005; Berneis et al., 1996; Douyon and Schteingart, 2002; Falorni et al., 1997; Friedman and Halaas, 1998; Gutin et al., 1999; Pilcova et al., 2003; Reiterer et al., 1999; Rubin et al., 2005; Sudi et al., 2001) and lower levels of human growth hormone (hGH) (Argente et al., 1997; Coutant et al., 1998; Douyon and Schteingart, 2002; Ghizzoni and Mastorakos, 2003; Rasmussen et al., 1995; Roemmich et al., 2005) than normal weight youth. These differences may help to explain why obese youth are at an increased risk for diseases such as type II diabetes and cardiovascular disease. However, relatively few studies have examined how changes in weight status are related to changes in these hormones in youth.

The relationship between changes in hormones and changes in weight status is best understood in leptin, as the majority of longitudinal studies have shown direct relationships between change in weight and change in leptin (Falorni et al., 1997; Gallistl et al., 2001; Geldszus et al., 1996; Lazzer, Vermorel, et al., 2005; Pilcova et al., 2003; Reiterer et al., 1999; Reinehr et al., 2005; Savoye et al., 2002; Sudi et al., 2001). Only one study was found that investigated the effects of change in weight status on change in cortisol in youth and these authors reported reductions in cortisol only in obese, insulin resistant children who lost a significant amount of weight (Reinehr and Andler, 2004a). Likewise, only one study of change in weight status and change in hGH in children was found, and they reported increased levels of hGH in prepubertal children, following a weight loss program (Argente et
Taken together, these studies demonstrate a trend for decreased leptin and cortisol and increased hGH with weight status reduction.

In addition to the influence of changes in weight status, changes in these hormones may influence each other. For example, research shows that leptin may inhibit the release of cortisol (Leal-Cerro et al., 2001; Wauters et al., 2000) and hGH (Carro et al., 1997; Coutant et al., 1998), while cortisol has been shown to stimulate the release of leptin (Berneis et al., 1996; Ghizzoni and Mastorakos, 2003; Leal-Cerro et al., 2001; Wauters et al., 2000) and inhibit the release of hGH (Barbarino et al., 1990; Ghizzoni et al., 1996). The effect of hGH on the release of leptin or cortisol is controversial, however. The interrelationships of these hormones have not been studied previously in youth undergoing changes in weight status. Therefore, the purposes of this study are: 1) to determine the relationship between changes in weight status and changes in leptin, cortisol and hGH, and 2) to determine the interrelationships among changes in leptin, cortisol and hGH in youth undergoing changes in weight status.

METHODS

Participants

A total of 120 participants, obtained from Cohort 5 of the Cardiovascular Health in Children III (CHIC) study, took part in data collection (J.S. Harrell, P.I.). Participants were selected from a larger cohort based upon their weight status at time 1 (t1) and two years later (t2), and the availability of stored blood samples. The mean age of participants in the original cohort was 9.6±1.1 years at t1 and 11.5±1.0 years at t2. Similarly, there were 50% females and 50% males in this cohort, with a racial distribution as follows: 53% Black, 36% White,
and 11% Other. The participants were selected and divided into the following four groups: NO (normal weight at \( t_1 \) and overweight at \( t_2 \)), NN (normal weight at both testing times), ON (overweight at \( t_1 \) and normal weight at \( t_2 \)), and OO (overweight at both testing times). For the purposes of our investigation, normal weight was defined as \(< 85^{th} \) and \( > 5^{th} \) BMI percentile, while overweight was \( \geq 85^{th} \) percentile for BMI (Centers for Disease Control and Prevention Growth Charts, 2000). The participants in the NN and OO groups were matched to those in the NO and ON groups, respectively, based on sex, race and pubertal status at \( t_1 \) since these variables are known to influence the outcome measures in this investigation. This was done by identifying all participants from the original cohort that were matched to individuals on these criteria, and then randomly selecting from those to comprise the NN and OO groups. The NO and NN groups had 35 participants each, while the OO and ON groups had 25 participants per group. A larger numbers of participants actually changed weight status over the two year period; however, the final groups included only those participants with complete data available for analysis.

**Procedures**

All methods were approved by the Institutional Review Board at the University of North Carolina at Chapel Hill. Data were collected at the participants’ school sites by trained research assistants (RA). Prior to data collection, parents signed an informed consent form, while the participants completed an assent form. Height was measured via a portable stadiometer (Perspective Enterprises, Portage, MI), while body mass was measured via an electronic scale (Model 2101KL, Healthometer Medical, Bridgewater, IL). These measurements were taken twice and averaged to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated from height and body mass (kg/m\(^2\)). Percent body
fat was calculated via the sum of skinfolds from the subscapula and triceps. Using Lange calipers (Cambridge Scientific, Cambridge, MD) these skinfolds were measured in triplicate, using the methods set forth by NHANES III (1974). These values were used to calculate percent body fat in equations specific for each sex, race and pubertal stage (Slaughter et al., 1988). Additionally, participants completed a pubertal development questionnaire, specific to each sex (Petersen et al., 1988).

All blood draws were taken in the morning, from 7 to 9am, after an overnight fast of 12 hours verified by the RA’s. The blood samples were centrifuged at 4°C, the plasma decanted and stored in vials. All samples were stored at -80°C until the time of analysis. Leptin, cortisol and human growth hormone (hGH) were measured from the stored plasma using radioimmunoassay (RIA) techniques. All samples were analyzed in duplicate, with the exception of three samples in the hGH assay that were completed with only one sample due to limited volume. Leptin and hGH were measured using mobile phase, double-antibody RIA kits (LINCO Research, St. Charles, MO; MP Biomedicals, Costa Mesa, CA). The sensitivity of these kits was 0.5 ng/mL for leptin and 0.31 ng/mL for hGH. Cortisol was assessed using solid phase, single-antibody RIA kits, with a sensitivity of 0.2 µg/dL (Diagnostic Products Corporation, Los Angeles, CA). The mean coefficients of variation (CV) for the leptin, hGH and cortisol assays were 8.1%, 7.2% and 6.4%, and the intraassay CVs were: 5.8%, 8.5% and 4.5%, respectively.

**Statistical Analysis**

In order to identify group differences at $t_1$, a one-way ANOVA was completed, comparing the following variables: age, pubertal stage, height, weight, BMI, BMI percentile, percent fat, leptin, cortisol, and hGH. Likewise, a Mantel-Haenszel chi-square was
computed to reveal any baseline group differences for race and sex. To quantify the amount of change in leptin, cortisol and hGH over the two year study period, change scores were calculated for each participant as follows: \((t_2 - t_1)\). These values were averaged for each group.

Analyses of covariance (ANCOVA) were conducted for each hormone to determine group differences in the amount of change after controlling for race, sex, and the hormone values at time 1. Separate ANCOVAs were computed for each of the following weight group comparisons: NO vs. ON, NN vs. NO and OO vs. ON. We included race as a covariate in the group comparisons since there was an unequal distribution of races in each group. Sex was also a covariate since it is known to influence resting levels of leptin in youth (Ahmed et al., 1999; Ankarberg-Lindgren et al., 2001; Falorni et al., 1997). Finally, initial examination of the data revealed that the majority of participants with elevated hGH levels were African American females, further supporting the need for race and sex as covariates in our analyses.

To determine the association among change in BMI percentile and changes in leptin, cortisol and hGH, partial Pearson correlations were calculated after controlling for race and sex. Partial Pearson correlations were also calculated to assess the strength of the interrelationships among the changes in leptin, cortisol and hGH, controlling for race and sex, and 95% confidence intervals were also computed. All statistical analyses were completed with SAS Statistical Software, Version 9.1 (Cary, NC) and the alpha level was equal to 0.05.
RESULTS

Participant Characteristics

The participant characteristics at t₁ and t₂ are shown in Table 1. At t₁, participants in the OO and ON groups had a larger mean BMI percentile than those in the NN and NO groups. There were group differences in race, such that the NN and NO groups had more Black participants than the ON and OO groups. Participants in the NO group were younger than participants in all other groups by approximately one year (p<0.05). Finally, the OO group had higher mean leptin values at t₁, compared to all other groups.

Group Comparisons of Changes in Leptin, Cortisol and hGH

There were significant differences between the NO and ON groups for changes in leptin and cortisol (p<0.001). As shown in Table 2, there was a large increase in leptin in the NO group, and a slight decrease for the ON group. For cortisol, the ON group increased slightly, while the NO group decreased by a larger magnitude. The change in hGH was nearly identical for the NO and ON groups, but in opposite directions.

To describe the changes associated with becoming overweight, we compared participants in the NO group to those in the NN group. There were significant differences for leptin, as the NO group had a greater increase compared to the NN group, as expected. Similarly, the NO group had a larger decline in cortisol, compared to the NN group. Finally, when comparing participants who became normal weight to those who remained overweight, the only significant group difference was in leptin as the OO group increased markedly, while the ON group declined.
Table 1. Participant characteristics (mean ± standard deviation), presented by weight status group at t₁ and t₂.

<table>
<thead>
<tr>
<th></th>
<th>NN</th>
<th>NO</th>
<th>ON</th>
<th>OO</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>35</td>
<td>35</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>9.8±0.9</td>
<td>11.7±0.9</td>
<td>10.2±1.0</td>
<td>10.1±1.1</td>
</tr>
<tr>
<td>Sex (females, males)</td>
<td>17, 18</td>
<td>14, 11</td>
<td>14, 11</td>
<td></td>
</tr>
<tr>
<td>Race (black, white, other)</td>
<td>21, 13, 1</td>
<td>8, 15, 2</td>
<td>8, 15, 2</td>
<td></td>
</tr>
<tr>
<td>Median Pubertal Stage (range)</td>
<td>2 (1-4)</td>
<td>2 (1-4)</td>
<td>2 (1-4)</td>
<td>2 (1-4)</td>
</tr>
<tr>
<td>Body Mass (kg)†</td>
<td>34.2±5.3</td>
<td>42.8±6.5</td>
<td>45.9±8.8</td>
<td>53.6±16.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>141.0±7.3</td>
<td>152.4±8.1</td>
<td>145.8±8.7</td>
<td>143.4±9.1</td>
</tr>
<tr>
<td>BMI (kg/m²)‡</td>
<td>17.1±1.6</td>
<td>18.4±1.7</td>
<td>21.4±2.3</td>
<td>25.7±5.3</td>
</tr>
<tr>
<td>BMI percentile**</td>
<td>50.8±24.4</td>
<td>51.8±23.1</td>
<td>75.3±9.3</td>
<td>95.3±3.9</td>
</tr>
<tr>
<td>Fat (%)‡</td>
<td>16.0±5.3</td>
<td>17.6±7.0</td>
<td>21.6±5.9</td>
<td>31.2±8.2</td>
</tr>
</tbody>
</table>

* p<0.05, NO < NN, ON and OO at baseline, † p<0.05, NN and NO < ON < OO at baseline
** p<0.05, NN < NO < ON and OO at baseline, ‡ p<0.05, NN < NO < ON < OO at baseline

Table 2. Leptin, cortisol and hGH at t₁ and t₂, and changes (t₂-t₁) in these hormones (mean ± standard deviation), presented by weight status group.

<table>
<thead>
<tr>
<th></th>
<th>NN</th>
<th>NO</th>
<th>ON</th>
<th>OO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/mL)*</td>
<td>1.7±1.1</td>
<td>3.1±2.0</td>
<td>12.2±7.6</td>
<td>15.8±11.7</td>
</tr>
<tr>
<td>Δ Leptin †</td>
<td>1.43±1.62</td>
<td>8.19±6.83</td>
<td>-1.35±4.88</td>
<td>6.68±11.87</td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
<td>14.1±5.6</td>
<td>12.0±4.5</td>
<td>13.5±5.2</td>
<td>12.2±6.2</td>
</tr>
<tr>
<td>Δ Cortisol ‡</td>
<td>-2.07±6.18</td>
<td>-4.91±7.18</td>
<td>1.63±5.23</td>
<td>-0.89±7.22</td>
</tr>
<tr>
<td>hGH (ng/mL)</td>
<td>2.1±3.7</td>
<td>4.0±4.6</td>
<td>3.9±4.8</td>
<td>1.9±2.6</td>
</tr>
<tr>
<td>Δ hGH</td>
<td>1.88±6.34</td>
<td>0.98±3.90</td>
<td>-0.79±6.03</td>
<td>1.35±3.64</td>
</tr>
</tbody>
</table>

* p<0.05, OO > ON, NO and NN at baseline; ON > NO at baseline
† p<0.05, NO > ON, NO > NN, OO > ON
‡ p<0.05, NN > NO, ON > NO and OO
**Change in BMI Percentile and Percent Change in Hormones**

The partial Pearson correlations between change in BMI percentile and changes in the hormones were weak to moderate in strength, yet all statistically significant. The correlations between change in BMI percentile and changes in leptin, cortisol and hGH were 0.39, -0.25 and 0.24, respectively, after adjusting for race and sex. The 95% confidence intervals for these relationships were: 0.22 to 0.53 for changes in leptin and BMI percentile, -0.07 to -0.41 for changes in cortisol and BMI percentile and 0.06 to 0.40 for changes in hGH and BMI percentile.

**Hormonal Interrelationships**

In general, the partial Pearson correlations between the changes in leptin, cortisol and hGH, after controlling for race and sex, were weak. The only statistically significant correlation was between the change in leptin and cortisol (r=-0.23, p<0.05). The correlations of the changes for hGH and either leptin or cortisol were 0.02 and -0.11, respectively (p>0.05).

**DISCUSSION**

**Interrelationships among Changes in Leptin, Cortisol and hGH**

In general, the interrelationships between changes in leptin, cortisol and hGH were weak. The only significant correlation was between the changes in leptin and cortisol (r=-0.23), after accounting for race and sex. This inverse relationship has not been reported previously in youth, and suggests that the large increases in leptin in youth who became overweight (NO) may be related to the decline in cortisol. Conversely, in the ON group, the slight decrease in leptin may be related to the slight increase in cortisol. However, previous
researchers reported positive relationships among these hormones in youth when comparing samples taken over the course of 24 hours (Ghizzoni and Mastorakos, 2003). These authors and other researchers noted that the relationship between leptin and cortisol release may be time-dependent, such that increases in leptin lead to increases in cortisol, following a lag time of several hours (Ghizzoni and Mastorakos, 2003; Koutkia et al., 2003). Our results may differ from their findings since we compared youth undergoing weight status change over a two year period, and used only one, early morning blood sample per measurement day.

We found no significant associations among changes in hGH and leptin, suggesting that changes in these hormones did not influence one another. In contrast to our findings, the aforementioned study by Ghizzoni and Mastorakos (2003) reported positive relationships between leptin and hGH in youth, with increases in hGH preceding leptin over the course of a day. Finally, there was no relationship between changes in cortisol and hGH, as demonstrated by the weak, nonsignificant correlation. This lack of association may be explained by the common influence of leptin on these hormones. Research in adults shows that the release of cortisol and hGH are regulated by leptin, not each other (Koutkia et al., 2003).

**Weight Status Group Comparisons**

*Leptin.* In general, our leptin values were similar to previous reports in normal (Brandao et al., 2003; Coutant et al., 1998; Reinehr et al., 2005) and obese (Aygun et al., 2005; Coutant et al., 1998; Reinehr et al., 2005; Rubin et al., 2005) youth of similar ages. When all participants were analyzed, the strongest association ($r=0.39$) was between change in BMI percentile and the change in leptin. However, when we compared the change in leptin according to weight status group, the greatest change in leptin was seen in participants
who became overweight (NO), and this change was significantly different from those who became normal weight (ON) or those who maintained a normal weight status (NN). These findings suggest that leptin was more sensitive to becoming overweight, rather than becoming normal weight, even when the magnitude of change in BMI percentile was the same (14 units).

The trends in leptin change of the ON and OO groups are similar to previous research by Reinehr et al. (2005). These authors found that overweight youth who had undergone substantial weight loss, defined as change in SDS-BMI $\geq 0.5$ in one year, had only a small decrease in leptin, whereas those who maintained their overweight status ($< 0.5$ change in SDS-BMI) had a significant rise in leptin over a one year period. Other studies have reported larger reductions in leptin in response to weight status reduction, which may be attributable to the greater starting values for leptin and percent body fat or BMI in these participants (Lazzer, Vermorel, et al., 2005; Pilcova et al., 2003; Reiterer et al., 1999). Additionally, the small change in leptin in the ON group in our study may be due to our classification of weight status. These participants were only moderately overweight at time 1, with a mean at the 89th BMI percentile; similarly, at time 2, these participants were at the very high end of the normal weight range according to BMI percentiles. If these participants started with a higher BMI percentile or ended with a lower BMI percentile, changes in leptin may have been observed. Finally, it is important to point out that changes in weight status in our investigation were not invoked by an intervention per se, and they occurred as a result of natural processes and behaviors over a two year period. Thus, the related changes in hormones may differ from studies that used a weight change intervention.
Cortisol. In general, the magnitudes of change in cortisol were small and the only group differences were between the NO and each of the ON and NN groups. Interestingly, the changes in the NO and ON groups were in opposite directions, and opposite of what we expected. These findings are somewhat puzzling, considering that the group means for cortisol were similar to previous research for both normal (Coutant et al., 1998; Reinehr and Andler, 2004a) and overweight youth (Coutant et al., 1998; Reinehr and Andler, 2004a; Rubin et al., 2005). Even when weight status was analyzed using the continuous variable of BMI percentile, an inverse relationship (r=-0.25, p<0.05) was found. Only one previous study was found that analyzed the effects of change in weight status on change in cortisol in youth, and their results were opposite of ours (Reiner and Andler, 2004a). These researchers reported reductions in cortisol after significant weight loss (≥0.5 standard deviation score for BMI) over a one year period, only in obese participants with insulin resistance (Reinehr and Andler, 2004a). Their results may have differed from our investigation due to the magnitude of weight change. While the actual change in weight or fat mass was not reported in their investigation, a change of ≥0.5 standard deviation scores for BMI was equal to a reduction in BMI of 3.3 units in a related study by these authors (Reinehr and Andler, 2004b). This change was much greater than the 0.9 unit reduction in BMI for the ON group in our investigation.

Several other factors may help to explain why the groups did not follow the trends that we anticipated. For example, despite the group differences in the change in cortisol, the actual changes in cortisol were small, and the standard deviations were large. This may be a reflection of the normal within day variation in cortisol due to its circadian pattern of release. In support of this notion, researchers have reported substantial differences in the circadian
pattern of cortisol release among youth (Knuttson et al., 1997). Additionally, while weight-related differences in cortisol may not be evident at rest, they may be present in response to a stressor such as exercise. For example, researchers found similar values for cortisol in normal weight and obese youth at rest, but the obese participants had a greater reduction in cortisol during and following peak exercise, compared to the normal weight youth (Eliakim et al., 2006). Similarly, these researchers noted trends for lower levels of catecholamines in obese youth at rest and during exercise and these hormones also influence cortisol (Eliakim et al., 2006).

**Human Growth Hormone.** The change in hGH did not differ among any of the four weight status groups (p>0.05), and the magnitude of change in hGH was quite small. Notably, the NO group increased slightly, while the ON group decreased by a similar amount over the two year study period. These results were not expected, as we hypothesized that hGH would increase in the ON group and decrease in the NO group. However, the mean hGH values were quite small, with very large standard deviations. These factors make it difficult to detect changes in response to weight status change. Also, our hGH values were slightly lower than previous reports for normal weight youth (Ballerini et al., 2004; Zakas et al., 1994) and slightly higher than reported values for obese youth (Argente et al., 1997; Ballerini et al., 2004; Eliakim et al., 2006). Taken together, these factors may explain why our results differed from our hypotheses.

The correlation between change in BMI percentile and change in hGH (r=0.24, p<0.05) suggests that there is a positive relationship, albeit weak, between weight status change and change in hGH. To our knowledge, no previous investigation has compared these changes in this population. However, when researchers studied postpubertal girls
(mean age 18.9 yrs), negative relationships were found between change in hGH and change in percent body fat or BMI (Kasa-Vubu et al., 2006). These differences may be attributable to the differences in hGH measurement. Kasa-Vubu et al. (2006) used mean hGH values, derived from measures taken throughout a 24-hour period, as opposed to the single morning sample used in our investigation.

**Strengths and Limitations**

The use of weight status groups to compare participants who became normal weight and participants who became overweight was a key strength in this study. This distinction is important when using longitudinal designs in children, since they may be gaining weight as they grow, but their BMI percentile and related weight status may actually be decreasing. Additionally, these classifications are commonly used by practitioners and researchers to delineate levels of disease risk and to make health recommendations. Another strength was the examination of natural changes in weight status, not changes in weight status that were induced by an intervention. This design improves the generalizability of our results, as the majority of youth are not involved in weight change interventions.

A limitation associated with this investigation is the variability in hormone levels. Leptin (Ankarberg-Lindgren et al., 2001; Kasu-Vibu et al., 2006; Licinio et al., 1997; Schoeller et al., 1997; Sinha et al., 1996;) and hGH (Argente et al., 1997; Bertherat et al., 1995; Rasmussen et al., 1995) are released in a pulsatile fashion and cortisol has a circadian rhythm (Hermida et al., 1999; Rich et al., 1992). By only obtaining one blood sample at each time point, it is difficult to determine if the hormone levels varied due to the time of day or other factors such as weight status. In an effort to reduce the variability introduced by daily hormone fluctuations, we standardized the time of day during which blood draws were taken.
Future investigations may benefit from repeated sampling of hormones throughout the day, however. Finally, we are unable to describe the temporal associations of changes in these variables and as a result, cannot conclude if changes in weight status preceded changes in leptin, cortisol and hGH, or vice versa.

**Summary**

The results of this study showed that changes in leptin, cortisol and hGH are more strongly associated with changes in BMI percentile than with changes in one other. The only significant hormonal interrelationship was between changes in leptin and cortisol ($r=-0.23$). Likewise, becoming overweight was associated with larger changes in leptin and cortisol than becoming normal weight and there were no group differences for change in hGH.
REFERENCES


CHAPTER FOUR
Manuscript Three

The Relationship between Changes in Leptin, Cortisol, and Human Growth Hormone with Changes in Insulin in Youth

Kristin S. Ondrak, Robert G. McMurray, Anthony C. Hackney, and Joanne S. Harrell
ABSTRACT

INTRODUCTION: The interrelationships among changes in weight status, insulin, leptin, cortisol and human growth hormone (hGH) are not well understood in youth. This investigation determined the association between changes in these variables in youth who underwent weight status increase, decrease or maintenance over two years. METHODS: Data was collected for 120 youth at baseline (9.8±1.0 years) and two years later. Participants were selected from a larger cohort to represent all scenarios of weight status: normal weight (>5th and <85th BMI percentile) or overweight (≥ 85th BMI percentile) at both time points, normal weight who became overweight and overweight who became normal weight. Plasma leptin, cortisol, hGH and insulin were measured via radioimmunoassay procedures. Multiple regression analyses were used to determine the association between insulin change and changes in BMI percentile, leptin, cortisol and hGH, and significant variables were entered into an overall model. RESULTS: The partial correlation, controlling for race and sex, between change in insulin and change in leptin was 0.29 (p<0.05). Change in insulin was not related to changes in cortisol (r=-0.08) or hGH (r=0.16) (p>0.05). In the multiple regression analyses, changes in BMI percentile, leptin and hGH accounted for significant amounts of variance in insulin change (p<0.05), with change in leptin accounting for the greatest amount of variance (9%), followed by change in BMI percentile (4%) (p<0.05). CONCLUSION: In a sample of youth who increased, decreased or maintained their weight status over two years, changes in insulin were most strongly associated with change in leptin, followed by change in BMI percentile. Supported by NINR #NR01-1837.
INTRODUCTION

In youth, research has shown that insulin levels are related to weight status, such that overweight youth have higher insulin levels (Lee et al., 2006; Reinehr et al., 2004). Weight status has also been shown to affect leptin, cortisol, and human growth hormone (hGH) (Cruz et al., 2005; Dominici and Turyn, 2002; Lazzer, Vermorel et al., 2005; Reinehr and Andler, 2004). Furthermore, researchers have identified relationships between these three hormones and circulating insulin levels in youth. The mechanisms linking leptin, cortisol, and hGH to insulin have been described using rodent models. For example, in mice and rats, leptin and cortisol have been shown to decrease insulin release (Cases et al., 2001; Delaunay et al., 1997; Zhao et al., 1998), whereas hGH stimulates insulin release (Smith, Elmendorf et al., 1997). Additionally, insulin has been shown to increase the release of leptin and decrease the release of cortisol and hGH (Barr et al., 1997; Boden et al., 1997; Havel, 2000; Havel, 2004; Jamieson et al., 1995; Kieffer and Habener, 2000; Lee et al., 2001; Wabitsch et al., 1996; Wauters et al., 2000). Taken together, these studies highlight the associations between leptin, cortisol and hGH with insulin; however, the longitudinal relationships between changes in these hormones and changes in insulin in youth are not well understood. Additionally, it remains unclear as to which of these hormones has the strongest relationship with changes in insulin in youth.

The purpose of this investigation was to examine the relationships between changes in these hormones with change in insulin in youth undergoing natural changes in weight status over a two year period. We expected that change in insulin would be positively correlated with changes in leptin and cortisol and negatively correlated to changes in hGH. Additionally, we hypothesized that each of the following would explain a significant portion
of the variance in change in insulin: changes in BMI percentile, leptin, cortisol, hGH. Finally, we expected changes in leptin to account for the greatest amount of variation in change in insulin.

METHODS

Data Collection Procedures

Participants were selected from the Cardiovascular Health in Children III (CHIC) study (J.S. Harrell, P.I.). A total of 120 children and adolescents took part in this study and data were obtained by trained research assistants at the participant’s schools at baseline, \( t_1 \), and two years later, \( t_2 \). The participants were selected to represent all possible scenarios of weight status change and maintenance: normal weight (\( >5^{th} \) and \( <85^{th} \) BMI percentile for age and sex) at both time points, overweight (\( \geq 85^{th} \) BMI percentile for age and sex) at both time points, normal weight at \( t_1 \) and overweight at \( t_2 \), and overweight at \( t_1 \) and normal weight at \( t_2 \). The participants signed assent forms and their parent(s) signed informed consent forms prior to data collection. All procedures were approved by the Institutional Review Board at the University of North Carolina at Chapel Hill.

Body mass and height were measured using an electronic scale (Model 2101KL, Healthometer Medical, Bridgewater, IL) and a stadiometer, respectively (Perspective Enterprises, Portage, MI). These measures were taken with the participant barefoot and measures were recorded to the nearest 0.1 kg and 0.1 cm, respectively. These measurements were taken twice and averaged and used to calculate body mass index (BMI, \( \text{kg/m}^2 \)). Research assistants also measured skinfold thickness at the triceps and subscapular sites, using procedures set forth by the National Health and Nutrition Examination Survey (1974).
Measurements were taken in triplicate using Lange calipers (Cambridge Scientific Instruments, Cambridge, MD) and means calculated for each site. Percent body fat was calculated using equations specific to each sex, pubertal stage and race (in males only) (Slaughter et al., 1988) and these values were used to calculate fat and fat free mass.

In addition, pubertal development (Roemmich et al., 2002) and aerobic power (McMurray et al, 2000) were measured since they have been shown to influence insulin in youth. Pubertal development was self-assessed via a questionnaire (Petersen et al., 1988), while aerobic power was predicted using a submaximal test performed on the cycle ergometer, the PWC\textsubscript{195} (McMurray et al, 1998). We chose to express aerobic power in ml/kg\textsubscript{FFM}/min in order to remove the influence of fat mass, and focus on the oxygen consumption of fat free mass since fat free mass is most related to insulin uptake. The following equation was used to calculate aerobic power in ml/kg\textsubscript{FFM}/min: \([\text{VO}_2 \text{ in ml/kg/min} \times \text{body mass in kg}] / \text{fat free mass in kg}\).

Finally, blood was obtained via standard venipuncture procedures between 7 and 9 am, following a verified 12 hour fast. The blood was centrifuged at 4°C in order to separate the plasma, which was subsequently stored at -80°C until it was analyzed. Insulin, leptin, cortisol and hGH were measured from the stored plasma using radioimmunoassay (RIA) procedures. In each assay, samples were analyzed in duplicate, with the exception of three samples in the hGH assay that were run as singlets due to limited plasma volumes. Insulin, leptin and hGH were analyzed using mobile phase, double-antibody RIA kits (insulin and leptin, LINCO Research, St. Charles, MO; hGH, MP Biomedicals, Costa Mesa, CA). The lowest detectable limits of these assays were 2 \(\mu\)U/mL, 0.5 ng/mL, and 0.31 ng/mL, respectively. Cortisol was measured using solid phase, single-antibody RIA kits, with a
lowest detectable limit of 0.2 $\mu$g/dL (Diagnostic Products Corporation, Los Angeles, CA). The mean coefficients of variation for the insulin, leptin, hGH, and cortisol assays were 8.0%, 8.1%, 7.2%, and 6.4%. Changes in leptin, cortisol, hGH and insulin were assessed using simple change scores: $t_2 - t_1$.

**Statistical Analysis**

To examine the relationships between two-year changes in insulin and changes in leptin, cortisol and hGH, partial Pearson correlations were computed after controlling for race and sex. Additionally, five multiple regression models were generated using the stepwise selection, to determine the amount of variance in insulin change. In each model, we controlled for variables known to influence insulin in youth including race, sex and changes in VO$_2$/kg$_{FFM}$ and pubertal status (Allen et al., 2007; Guzzaloni et al., 2002). In the first model, the independent variable of interest was change in BMI percentile. The second regression model was identical to the first model, with the addition of the independent variable change in leptin. The third and fourth models used change in cortisol and change in hGH, respectively, in place of change in leptin as the independent variables of interest. The final model was generated to determine which variable explained the greatest amount of variance in insulin change. It included changes in BMI percentile, leptin and hGH, since these variables were significant in their own respective models.

Finally, collinearity diagnostics were calculated to check for collinearity among the independent variables in the regression models. According to the recommendations of Belsley (1980), variables would be considered collinear if they had a high condition index (>30) and at least two variance proportions that are >0.5. Using these criteria, no variables
RESULTS

The mean hormonal concentrations at $t_1$ and $t_2$ are displayed in Table 1. In general, height, body mass and pubertal development increased over the two year period, as expected in youth. There were only small increases in percent body fat, BMI, BMI percentile and aerobic power (ml/kg_FFM/min). With regard to the hormones, insulin did not change, leptin and hGH increased by 63% and 38%, respectively, while cortisol declined by 14%. The partial Pearson correlations between the two-year changes in insulin and changes in leptin, cortisol and hGH, controlling for race and sex, were 0.29, -0.08, and 0.16, respectively. The only significant ($p<0.05$) correlation was between changes in insulin and leptin.

The results of the multiple regression analyses, controlling for race, sex and changes in VO$_2$/kg_FFM and pubertal status, are summarized in Table 2. While each of the five regression models was statistically significant, the total amount of variance in insulin change explained by the independent variables was small, ranging from 10 to 18%. In the first model, change in BMI percentile accounted for 6% of the variance in insulin change. In model 2, after the addition of change in leptin (partial $R^2$ =0.09), the relationship between change in BMI percentile and change in insulin was no longer significant (partial $R^2$ = 0.02, $p>0.05$). In the third model, change in cortisol was not a significant independent variable, and as a result, it was not added to the final model. Change in hGH was a significant variable in Model 4, accounting for 5% of the variance in change in insulin. In the final model, change in leptin explained the greatest amount of variance (9%) in change in insulin,
followed by change in BMI percentile (4%) and, although not statistically significant, change in hGH (3%).

**Table 1.** Participant characteristics (mean ± standard deviation), and insulin, leptin, cortisol and hGH at $t_1$ and $t_2$.

<table>
<thead>
<tr>
<th></th>
<th>$t_1$</th>
<th>$t_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>9.8±1.0</td>
<td>11.6±1.1</td>
</tr>
<tr>
<td><strong>Median Pubertal Stage (range)</strong></td>
<td>2 (1-4)</td>
<td>3 (1-5)</td>
</tr>
<tr>
<td><strong>Fat (%)</strong></td>
<td>22.7±8.2</td>
<td>24.0±9.2</td>
</tr>
<tr>
<td><strong>Body Mass (kg)</strong></td>
<td>40.9±12.2</td>
<td>51.4±13.1</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>141.3±8.8</td>
<td>152.8±9.5</td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>20.2±4.2</td>
<td>21.9±4.4</td>
</tr>
<tr>
<td><strong>BMI percentile</strong></td>
<td>75.4±22.2</td>
<td>76.8±21.9</td>
</tr>
<tr>
<td><strong>Aerobic Power (ml/kgFFM/min)</strong></td>
<td>47.4±11.4</td>
<td>48.9±11.0</td>
</tr>
<tr>
<td><strong>Insulin (µU/mL)</strong></td>
<td>14.8±8.8</td>
<td>14.7±9.4</td>
</tr>
<tr>
<td><strong>Leptin (ng/mL)</strong></td>
<td>6.2±7.3</td>
<td>10.1±10.9</td>
</tr>
<tr>
<td><strong>Cortisol (µg/dL)</strong></td>
<td>13.8±5.6</td>
<td>11.9±5.0</td>
</tr>
<tr>
<td><strong>hGH (ng/mL)</strong></td>
<td>2.4±3.5</td>
<td>3.3±4.0</td>
</tr>
</tbody>
</table>
Table 2. Multiple regression models of change in insulin with respect to changes in BMI percentile, leptin, cortisol and hGH, accounting for sex, race and changes in VO$_2$/kgFFM and pubertal status.

<table>
<thead>
<tr>
<th>Model</th>
<th>Partial $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: $\Delta$ BMI %tile</td>
<td>$\Delta$ BMI %tile $0.06^*$</td>
</tr>
<tr>
<td>Model 2: $\Delta$ BMI %tile + $\Delta$ Leptin</td>
<td>$\Delta$ Leptin $0.09^*$</td>
</tr>
<tr>
<td>Model 3: $\Delta$ BMI %tile + $\Delta$ Cortisol</td>
<td>$\Delta$ BMI %tile $0.06^*$</td>
</tr>
<tr>
<td>Model 4: $\Delta$ BMI %tile + $\Delta$ hGH</td>
<td>$\Delta$ hGH $0.05^*$</td>
</tr>
<tr>
<td>Model 5: $\Delta$ BMI %tile + $\Delta$ Leptin + $\Delta$ hGH</td>
<td>$\Delta$ Leptin $0.09^*$</td>
</tr>
</tbody>
</table>

* $p<0.05$

DISCUSSION

This investigation appears to be the first to explore the relationships between longitudinal changes in insulin and changes in leptin, cortisol and hGH in a sample of youth who underwent natural changes in, or maintenance, of weight status. Our results showed that of the three hormones, changes in leptin had the strongest association with changes in insulin. Similarly, change in leptin accounted for the greatest amount of variance in insulin change, independent of changes in BMI percentile. When these analyses were repeated using changes in percent body fat based on the sum of skinfolds, in place of change in BMI percentile, similar results were found, although the relationships were slightly weaker.
In this sample of youth, changes in insulin over the two-year observation period were positively associated with changes in leptin ($r=0.29$), when controlling for race and sex. This relationship is in agreement with a previous longitudinal study of obese youth after a one-year weight loss intervention (Reinehr et al., 2005). It is important to note that in our investigation, no intervention was used to invoke changes in BMI percentile, and we assume that they occurred as a result of normal processes. The significant correlation underscores the relationship between changes in insulin and leptin, since it remains even in the absence of a structured weight change intervention.

Cross-sectional studies in youth have also identified significant associations between insulin and leptin, even after adjusting for percent body fat ($r=0.18\text{-}0.24$) (Steinberger et al., 2003). The association between changes in leptin and insulin was also evident in the multiple regression models (Table 2). In these analyses, change in leptin accounted for a greater amount of variance in change in insulin than did changes in either BMI percentile or the other hormones. This novel finding lends further support to the association between change in leptin and change in insulin, independent of changes in BMI percentile in youth. This may be attributable to large increase in leptin (63%) that occurred over the two year study period, compared to the small increase BMI percentile (2%). The increase in leptin may be explained by concurrent rise in fat mass of 33%, since leptin is released from adipocytes (Friedman and Halaas, 1998). Researchers have proposed a theoretical model of the relationship between insulin, leptin and adipose tissue, termed the “adipo-insul ar” axis, which may help to explain our findings (Fehmann et al., 1997). In this axis, insulin is thought to increase the release of leptin from adipose tissue, while leptin decreases the release of insulin. The lack of change in insulin in the current investigation may be partially
explained by an inhibition of its release by increased leptin. Viewed collectively, our findings provide support for an association between changes in leptin and insulin in youth.

In the correlational analyses, changes in insulin were not significantly associated with changes in either cortisol or hGH. Similarly, change in cortisol was not related to change in insulin in the regression models. However, change in hGH was significant in the regression analysis explaining 5% of the variance in insulin change, after accounting for sex and change in BMI percentile. This suggests that changes in hGH and insulin are related, but their relationship is only evident after change in BMI percentile was considered. Finally, when change in leptin was included in the analysis (model 5), change in hGH was no longer significant, indicating that change in hGH had a weaker relationship with change in insulin than did change in leptin. Another possible explanation for this finding is that hGH and leptin are related in some way. For example, research shows that leptin can inhibit hGH release (Carro et al., 1997; Coutant et al., 1998). Conversely, research on the effects of hGH on leptin release are mixed as some authors reported that hGH stimulates leptin release (Fain and Bahouth, 2000; Ghizzoni et al., 2001), while others found that hGH inhibits the release of leptin (Isozaki et al., 1999; Wauters et al., 2000).

While there are no other longitudinal investigations in youth to which we can compare our results, studies using repeated sampling techniques over the course of one day may provide some explanation for our findings. Researchers have reported evidence of a time-dependent relationship of hormonal release in adults, such that insulin precedes leptin, which then influences the release of cortisol and hGH (Koutkia et al., 2003; Wagner et al., 2000). Taken together, these findings highlight the potential role of leptin as an intermediate in the relationship between insulin and cortisol or hGH. However, even if these relationships
exist in youth, the hormonal changes that occur over the course of two years may be
governed by additional factors beyond the scope of this study. Some possibilities include
growth, genetic factors, chromium intake (Anderson et al., 1997), cytokines such as TNF-α,
triglycerides, or HDL-cholesterol (Lichnovská et al., 2002).

Although a previous study in youth reported a significant, inverse relationship
between aerobic power per kilogram lean body mass and insulin (Allen et al., 2007), we
failed to detect an association among changes in VO$_2$/kgFFM and insulin in our regression
analyses. This may be because the participants in the Allen et al. (2007) investigation were
all obese (BMI >95$^{th}$ percentile) and the study was assessing cross-sectional relationships.
Also, the two-year changes in VO$_2$/kgFFM and fat free mass in our study were relatively small
(3% and 24%, respectively) considering that the participants were growing, which may
explain why we did not detect associations in their change scores in relation to insulin
change. In addition to aerobic power, insulin levels are related to pubertal status in youth.
For example, researchers have reported significantly higher insulin levels for youth in
pubertal stages 2-5, compared to the prepubertal participants in stage 1 (Guzzaloni et al.,
2002; Sudi et al., 2000). However, change in pubertal status did not influence change in
insulin in our regression models and this finding is likely due to the small changes in pubertal
status in our population, as 83% of the participants had either no change or an advance of one
pubertal stage over the two year study period. In addition, only 25% of the participants were
prepubertal at $t_1$, and as a result, the increase in insulin often reported following stage 1 has
likely already occurred. Taken together, our results show that despite the relationships
between insulin and either aerobic power or pubertal status demonstrated in previous cross-
sectional research, changes in these variables may not be significantly related to changes in insulin.

There were a number of strengths and weaknesses in this investigation. Our design was unique as it examined normal changes in weight status, as opposed to those caused by an intervention. Additionally, the inclusion of youth who were normal weight and became overweight two years later adds valuable information to the literature in this area. The number of youth who increase their weight status and become overweight is extremely high, yet few studies have examined them longitudinally. Finally, by also studying youth who decreased or maintained their weight status, we have representation from all possible scenarios of weight status change in youth. A key limitation was the use of single blood samples obtained at baseline and follow-up. The circulating levels of these hormones vary across the day, and multiple samples would have allowed us to correct for these changes.

In conclusion, we found positive associations between changes in leptin and changes in insulin in a sample of youth who increased, decreased or maintained their weight status over a two year period. Additionally, changes in insulin were related to changes in leptin, even after controlling for changes in BMI percentile, further emphasizing the association between leptin and insulin in youth. Changes in hGH were marginally related to change in insulin, after controlling for change in BMI percentile, however, insulin change was not related to cortisol change.


CHAPTER FIVE
Research Synthesis

Major Findings

The results of these investigations have revealed several important relationships among changes in weight status and changes in leptin, cortisol, human growth hormone (hGH) and insulin in youth. First, significant associations (r=0.26) were found between changes in BMI percentile and percent change in insulin resistance (HOMA-IR) when all participants were considered. The strength of this association increased (r=0.41) when only participants who underwent changes in weight status were included. Similar correlations were found when using percent change in insulin in place of HOMA-IR, but this was not the case when percent change in glucose was considered. These results demonstrate that changes in insulin, rather than glucose, were driving the changes in HOMA-IR. In the multiple regression analyses, change in BMI percentile was a significant predictor of change in insulin, accounting for 2 to 6% of its variance. Taken together, these results underscore the positive association between changes in weight status and changes in insulin or insulin resistance in youth.

In addition to analyzing changes in weight status using the continuous variable of BMI percentile, unique information was also gained by comparing changes in HOMA-IR between the four weight status groups. A key finding of these comparisons was that moving from normal weight to overweight was associated with larger changes in HOMA-IR (50%) than moving from overweight to normal weight status (-8%), even when the magnitude of
change in BMI percentile was identical. This finding highlights the importance of preventing youth from becoming overweight and demonstrates the difficulty in reducing insulin resistance even in the presence of weight status reduction.

The correlations between change in weight status and changes in the leptin, cortisol and hGH, controlling for race and sex, were significant but weak: change in BMI percentile and change in leptin (r=0.39), cortisol (r=-0.25) and hGH (r=0.24). By assessing group differences in these relationships, it was found that, compared to participants who were overweight and became normal weight, participants who were normal weight and became overweight had significantly greater increases in leptin (8.2 ng/mL vs. -1.4 ng/mL, respectively) and significantly greater decreases in cortisol (-4.9 µg/dL vs. 1.6 µg/dL, respectively). This again demonstrates that in general, becoming overweight is associated with larger changes compared to overweight youth becoming normal weight. However, this trend did not continue for changes in hGH, as no group differences were detected and the mean amounts of change were quite small (-0.8 ng/mL to 1.9 ng/mL).

Another aim of this investigation was to examine the interrelationships between changes in the leptin, cortisol and hGH and subsequently, the relationship between changes in each of these hormones with changes in insulin. In all of these comparisons, adjustments were made for race and sex since they are known to influence these hormones. The only significant hormonal interrelationship was found between change in leptin and change in cortisol and this association was inverse (r=-0.23). When examining the relationship between changes in insulin and changes in the hormones, the only significant association was between changes in leptin and insulin (r=0.29). The relationship between changes in leptin and insulin was also supported in the multiple regression models. In these analyses, the
amount of variance in insulin change explained by each of the following variables was assessed: changes in BMI percentile, leptin, cortisol and hGH, after controlling for race, sex, and changes in pubertal status and aerobic power per kg fat free mass. Changes in BMI percentile, leptin, and hGH were all significant variables in their own regression models. In the overall model, change in leptin accounted for the greatest amount of variance in change in insulin (partial $R^2 = 0.09$), while change in BMI percentile accounted for 4% of the variance. In general, however, the total amount of variance in insulin change explained by these independent variables was small (10 to 18%), suggesting that other factors contribute to the alterations in insulin in these youth.

**Significance of the Study and Implications of Results**

Current estimates show that 2 million adolescents have pre-diabetes, a precursor to type II diabetes, placing this disease as a major health concern for today’s youth (ADA website, 2007). During pre-diabetes, IR develops and indicates progress towards type II diabetes. An important, modifiable risk factor associated with IR in youth is weight status, specifically overweight status. Previous cross-sectional research shows that weight status and insulin are also related to hormones including leptin, cortisol and hGH in youth (Aygun et al., 2005; Casanueva et al., 1998; Coutant et al., 1998; Huang et al., 2004). The current study adds valuable, longitudinal data to the literature on these topics by determining if changes in weight status are related to changes in these hormones, and subsequently, if alterations in these hormones are related to insulin changes in youth. This research focuses on the associations between these variables in youth who underwent natural increases, decreases or maintenance of their weight status over a two year study period.
One of the major findings of the current investigation was that participants who were normal weight and became overweight had larger changes in IR and leptin than participants who were overweight and became normal weight in the two year study period. The relatively smaller changes in IR and leptin with weight status reduction suggest that a true reversal in these variables may take a longer period of time or may not be possible, as other factors may affect the cell’s sensitivity to insulin. Factors related to IR include genetics, inflammation, mitochondrial dysfunction and disruptions in the PI3-kinase signaling cascade (Krook et al., 2004, Morino et al., 2006; Petersen and Shulman, 2006, Shepherd et al., 2005, Wisse, 2004). Altogether, these findings imply that it is more beneficial to prevent youth from becoming overweight than attempting to reverse the negative consequences of being overweight. As a result, practitioners should emphasize the importance of maintaining a normal weight status.

Additionally, we found that of the three hormones, change in leptin had the strongest relationship with changes in insulin and changes in weight status, and both associations were positive. These results were expected, as previous studies in youth have reported positive relationships between change in leptin and changes in weight status or insulin (Gallistl et al., 2001; Lazzer, Vermorel, et al., 2005; Reineher, Kratzsch et al., 2005; Reiterer et al., 1999; Savoye et al., 2002; Sudi et al., 2001). However, the present study appears to be the first to report that changes in leptin accounted for a larger amount of variance in insulin change, than did changes in BMI percentile. This may be explained by several factors. First, the mean change in leptin was much larger than the mean change in BMI percentile (63% vs. 2%, respectively). The large increase in leptin is likely due to the 33% increase in fat mass that occurred over the two year study period, since leptin is released from adipocytes (Friedman and Halaas, 1998). In support of this finding, researchers have proposed a theoretical model
of the relationship between insulin, leptin and adipose tissue, termed the “adipo-insular” axis (Fehmann et al., 1997). In this model, insulin increases the release of leptin from adipose tissue, and leptin decreases the release of insulin. Thus, the lack of change in insulin may be partially attributable to an inhibition of its release by increased levels of leptin. Altogether, these results demonstrate that leptin is a key factor related to insulin and insulin resistance in youth.

The relatively weak relationships between changes in weight status and insulin with changes in both cortisol and hGH may be explained by several factors. First, the magnitude of change in these hormones was quite small and the standard deviations were large, making it difficult to detect any relationships among their change scores. Second, changes in cortisol and hGH did not follow the group related trends that we had expected. For example, one of the hypotheses was that cortisol would increase in participants who increased their weights status, and decrease in those who reduced their weight status; however, the opposite occurred. Likewise, the literature suggested that hGH would decrease in participants who became overweight and decrease when overweight youth normalized their weight, but the opposite occurred. Also, in the case of hGH, the values at both time points were slightly lower than expected in the normal weight youth (Ballerini et al., 2004; Zakas et al., 1994) and slightly higher than expected in the overweight youth (Argente et al., 1997; Ballerini et al., 2004; Eliakim et al., 2006). Finally, the interpretation of the data is complicated by the issue of hormonal fluctuations throughout the day, since only one blood sample per day was obtained.

With regard to the interrelationships among change in leptin, cortisol and hGH, our results also showed that these associations were weak, with the exception of a significant,
inverse relationship between changes in leptin and cortisol. A possible explanation for these findings is that other factors have a stronger influence on these hormonal changes, than the hormones did on one another. Some possibilities include race, sex, pubertal status, rate of growth, genetic factors, time of day, or the common influence of weight status on these hormones. Adjustments were made to account for the influence of these variables when possible. For example, race and sex were considered in the correlation analyses, and the time of day during which the blood draws were taken was standardized across all participants. Finally, the changes in pubertal status were quite small and similar among participants, and did not appear to impact these relationships.

**Summary of Research Hypotheses**

**Hypothesis 1a.** Insulin resistance will increase more in participants who were normal weight at $t_1$ and overweight at $t_2$ (NO) than in participants who were normal weight at both time points (NN).  **Result:** Supported

**Hypothesis 1b.** Insulin resistance will decrease more in participants who were overweight at $t_1$ and normal weight at $t_2$ (ON) than in participants who were overweight at both time points (OO).  **Result:** Rejected

**Hypothesis 1c.** Change ($t_2$-$t_1$) in BMI percentile will be positively related to change in insulin resistance. **Result:** Supported
**Hypothesis 2a.** Participants who were normal weight at $t_1$ and overweight at $t_2$ (NO) will have greater increases in leptin and cortisol and greater decreases in hGH compared to participants who were normal weight at both time points (NN). **Result:** Supported (leptin), Rejected (cortisol and hGH)

**Hypothesis 2b.** Participants who were overweight at $t_1$ and normal weight at $t_2$ (ON) will have greater decreases in leptin and cortisol and greater increases in hGH compared to participants who were overweight at both time points (OO). **Result:** Supported (leptin), Rejected (cortisol and hGH)

**Hypothesis 2c.** Change in BMI percentile will be positively related to change in leptin and cortisol and negatively related to change in hGH. **Result:** Supported (leptin), Rejected (cortisol and hGH)

**Hypothesis 3a.** Change in leptin and cortisol will be positively correlated to each other and negatively correlated to change in hGH. **Result:** Rejected

**Hypothesis 3b.** Change in insulin will be positively correlated with changes in leptin and cortisol and negatively correlated to change in hGH. **Result:** Supported (insulin and leptin), Rejected (insulin and cortisol, insulin and hGH)

**Hypothesis 4a.** Change in BMI percentile will explain a significant portion of the variance in change in insulin. **Result:** Supported
Hypothesis 4b. Change in leptin will explain a significant portion of the variance in change in insulin. Result: Supported

Hypothesis 4c. Change in cortisol will explain a significant portion of the variance in change in insulin. Result: Rejected

Hypothesis 4d. Change in hGH will explain a significant portion of the variance in change in insulin. Result: Supported

Hypothesis 4e. Change in leptin will account for the greatest amount of variance in change in insulin. Result: Supported

Strengths and Limitations

After reviewing the literature, it is evident that the majority of studies have focused on the effects of weight loss or weight reduction, rather than maintenance or gain. However, the current investigation evaluated the effects of all three: increase, decrease, and maintenance of weight status. Additionally, we chose to study youth undergoing natural, unprovoked changes in weight status in order to understand the relationships between these changes with changes in hormones and insulin resistance. These design features are key strengths of the current investigation and our results provide valuable comparisons that can be used to guide future studies.

Limitations of the current studies include the use of HOMA rather than a gold standard clamp study for the estimation of insulin resistance (IR) in youth. However, the
HOMA has been shown to be a valid marker of IR in obese youth, as a correlation of -0.89 was reported with a frequently sampled intravenous glucose tolerance test (Conwell et al., 2004). Another study of obese youth reported strong diagnostic statistics for HOMA-IR (sensitivity = 76% and specificity = 66%), providing further rationale for its use (Keskin et al., 2005). The within and between day variation in leptin, cortisol and hGH make it difficult to determine if the changes observed were true changes or simply variations within or between days. We attempted to minimize the between day variability by standardizing the time of day at which the blood draws were taken, between 7 and 9 am. The results of studies in which multiple blood samples were obtained over a 24-hour period show that this window of time is several hours after the peak release of leptin (Ankarberg-Lindgren et al., 2001) and hGH (Hermida et al., 1999), which occur sometime after midnight and decrease slightly during the morning hours. Alternatively, cortisol has been shown to peak in the early morning, ~6 am, (Knutsson et al., 1997), or shortly thereafter, ~10 am, (Hermida et al., 1999) in youth, followed by a decline throughout the day. As a result, our values were likely similar to the peak value for cortisol, but less than the peak values for leptin and hGH. However, this pattern was consistent for both baseline and follow-up measurements. Finally, these blood samples were obtained following a 12-hour fast, in order to minimize the effects of meals and metabolism and this practice is common throughout hormonal research on these topics.

**Future Research**

If given the opportunity to advance and improve upon these studies, additional participants would be selected to represent minority populations such as African Americans,
Hispanics, Asian Americans and American Indians. These populations have the highest incidence rate of type II diabetes (Dabelea et al., 2007). In addition, Non-Hispanic Blacks and Mexican Americans were found to have the highest prevalence of overweight in children and adolescents in the United States (Ogden et al., 2007). Additionally, obtaining multiple blood samples throughout the day and using the mean hormonal values for all comparisons would be a better approach to the study of this topic. Finally, identifying the threshold of mass or fat mass loss needed to reduce IR in youth would be of interest, since the current investigation showed that changing weight status categories from overweight to normal weight only resulted in small reductions in IR. This would aid in the development of recommendations for fat or mass loss for youth who have increased fasting insulin levels or are pre-diabetic.
APPENDIX A
Literature Review

INTRODUCTION

The following literature review begins with a discussion of insulin and three hormones related to insulin resistance: leptin, cortisol and human growth hormone. In this section, the function of each hormone is reviewed, as well as the effect of obesity, the effect of change in weight and the pathway from each hormone to insulin resistance. Next, the interactions among the hormones and with insulin are highlighted. Finally, the review concludes with a summary of the relationship between fitness and insulin resistance.

HORMONES RELATED TO INSULIN RESISTANCE

Insulin

Function

Insulin is an anabolic hormone released from the beta cells of the pancreas in response to high levels of glucose in the blood (Cosford, 1999). It binds with its cell membrane receptor and begins a cascade of intracellular events that activate insulin receptor substrate (IRS) complexes and PI 3-kinase (PI3-k) which leads to translocation of GLUT transporters to the cell membrane (Havel, 2000; Krook et al., 2004; Porte et al., 2005; Whiteman et al., 2002). The GLUT transporters increase the uptake of glucose into cells, thus lowering blood glucose (Havel, 2000; Krook et al., 2004; Whiteman et al., 2002). Insulin also decreases glucose production by slowing the rates of glycogenolysis and gluconeogenesis in the liver and stimulating the production of glycogen from glucose (glycogenesis) (Cosford, 1999; Holt et al., 2003; Jeffcoate, 2002). Insulin is also involved in fat metabolism as it decreases the rate lipolysis and increases LPL activity, which aids in the
breakdown of circulating triglycerides (Andrews and Walker, 1999; Cosford, 1999; Holt et al., 2003; Jeffcoate, 2002; Lee et al., 1999). This promotes free fatty acid (FFA) entry into adipocytes and subsequent lipogenesis (Havel, 2000). Conversely, high levels of FFA stimulate the release of insulin from the beta cells (Lee et al., 1999). Insulin also decreases the rate of ketogenesis and increases protein synthesis from amino acids.

**Insulin Resistance**

Insulin resistance (IR), the inverse of insulin sensitivity, occurs when an individual becomes unresponsive to insulin’s actions. It is manifest through increased levels of blood glucose and circulating FFAs (Jeffcoate, 2002). Several mechanisms contribute to the development of IR including intracellular lipid accumulation, mitochondrial dysfunction, disruptions in the PI3-kinase signaling cascade, increased PKC activity, puberty and inflammation. Additionally, three hormones, leptin, cortisol and hGH contribute to IR. The mechanisms of these pathways are described in detail in each respective hormonal section of this review.

The accumulation of lipids within cells occurs due to increases in plasma fatty acids, diacylglycerol, fatty acyl coenzyme A and protein kinase C (PKC) levels within the cell (Dyck, 2005; Dyck et al., 2006; Morino et al., 2006; Petersen and Shulman, 2006). As a result, activity of the serine/threonine kinase pathway increases and the actions of insulin and the related IRS cascade are down-regulated. This decreases the activity of PI3-kinase and leads to reduced glycogenesis in the liver, increased gluconeogenesis in the liver and decreased GLUT-4 activity in muscle (Petersen and Shulman, 2006); all of which contribute to IR. Some researchers have suggested that mitochondrial dysfunction may account for the gains in intracellular lipids and related IR (Morino et al., 2006; Petersen and Shulman, 2006).
Regardless of the mechanism, research has shown that insulin resistant adults had nearly twice the amount of plasma free fatty acids compared to normal, insulin sensitive individuals, when matched for BMI (Bluher et al., 2001). Additionally, IR may develop in response to dysfunctions in the IRS and PI3-kinase signaling pathway (Krook et al., 2004; Shepherd, 2005). Specifically, SOCs proteins (suppressor of cytokine signaling) can inhibit insulin’s intracellular signal, thus blocking the action of insulin within the cell (Shepherd, 2005). Finally, research has shown that increased activity of PKC phosphorylates the IRS in muscle cells (Dohm, 2001). This in turn decreases the activity of tyrosine kinase and contributes to IR.

IR increases at the onset of and during puberty (ADA, 2000; Cruz et al., 2005; Guzzaloni et al., 2002; Roemmich et al., 2002; Sudi et al., 2000). This alteration in IR may be attributed to puberty-related gains in fat mass and hormones such as leptin or growth hormone (ADA, 2000; Roemmich et al., 2002). Finally, obesity-related IR has also been attributed to inflammation (Wisse, 2004). While the mechanisms are not fully understood, recent advances have shown that adipose tissue releases cytokines and hormones, which increase inflammation and impair the IRS signaling cascade and subsequently increase IR (Wisse, 2004).

Several methods are used to quantify IR, the most common of which is the homeostasis model of assessment: (HOMA-IR) (Matthews et al., 1985). The HOMA-IR was validated against the criterion method of a frequently sampled intravenous glucose tolerance test (FSIVGTT) in a group of obese children and a strong correlation was produced (r = -0.89) (Conwell et al., 2004). Similarly, HOMA-IR has been shown to be the most reliable measure of insulin resistance in obese youth, with a sensitivity of 76% and specificity of 66%
(Keskin et al., 2005). Mean HOMA-IR values for these youth (ages 8 to 17 years) ranged from 4.34 to 5.48 (Conwell et al., 2004). Researchers have reported significant correlations between HOMA-IR and measures of fasting blood glucose, insulin, insulin’s response to glucose and insulin sensitivity (0.26, 0.59, 0.50, -0.41, respectively) in a large sample of obese youth (Guzzaloni et al., 2002). Mean values of HOMA-IR reported in their investigation ranged from ~2.2 to 4.0 (Guzzaloni et al., 2002). Some authors have used a cut-point of HOMA >4 to indicate IR and ≤4 to represent no insulin resistance (Reinehr and Andler, 2004a). Other researchers have identified 3.16, 3.29 and 4.39 as cut-points for IR, however (Keskin et al., 2005; Lee, Okumura, et al., 2006).

Finally, research in adults shows that the coefficients of variation in HOMA-IR are relatively small. For example, they were 9.4% and 7.8% in diabetic and nondiabetic adults, respectively, when comparing three tests on the same day (Bonora et al., 2000). The coefficients increased slightly when HOMA-IR values from consecutive days were compared; 13.8% and 11.2% in diabetic and nondiabetic adults, respectively (Bonora et al., 2000).

Figure 2 illustrates the pathways between leptin, cortisol, hGH and insulin resistance. The relationship between weight status and each of these hormones, as well as their relation to insulin resistance are discussed in detail in each respective hormone section.
Numerous studies have reported that insulin levels are positively related to measures of adiposity in youth (Argente et al., 1997; Bacha et al., 2006; Berneis et al., 1996; Gower et al., 1999; Johnson et al., 2001; Lee, Okumura, et al., 2006; Reinehr et al., 2004; Roemmich et al., 2002; Sudi et al., 2000; Young-Hyman et al., 2001). A study of 6-14 year old youth found that average fasting insulin levels were more than five times greater in obese compared to normal weight youth (2.6 vs. 13.7, p<0.001) (Reinehr et al., 2004). Significant and positive relationships were also reported between fasting insulin levels and increases in fat mass during a longitudinal study of ~8 year old children (Johnson et al., 2001). These researchers reported that the children with higher fasting insulin at the start of the study showed the greatest increases in fat mass, even after controlling for baseline levels of body fat (Johnson et al., 2001). Inverse associations have also been reported between elevated fat mass and reduced insulin sensitivity in children (Gower et al., 1999; Johnson et al., 2001;
Young-Hyman et al., 2001). Thus, obesity is associated with elevations in insulin and IR, increasing the risk of type II diabetes in obese youth.

Girod and Brotman (2003) presented a theoretical mechanism by which obesity contributes to hyperinsulinemia, which in turn, promotes the accumulation of fat mass. They identified several factors contributing to this cycle: high levels of glucocorticoids, low levels of hGH and increased lipolysis and FFA flux. Normally, insulin suppresses lipolysis and the related FFA release; however lipolysis is never completely inhibited and small amounts of FFA are released (Girod and Brotman, 2003). In an obese individual, the FFA release due to lipolysis is increased since their fat mass is greater than someone of normal weight. This abundance of FFA has been shown to increase the oxidation of fats, reduce the oxidation of glucose, and increase liver gluconeogenesis and glucose production (Girod and Brotman, 2003; Jensen, 2006). As a result, increasing amounts of insulin are released from the pancreas and insulin resistance develops in liver and muscle cells (Girod and Brotman, 2003; Jensen, 2006). The associated hyperinsulinemia contributes to increased glucocorticoids, reduced hGH and further increases in fat mass. This model presents obesity and hyperinsulinemia as a cycle that once initiated, perpetuates itself. This demonstrates the importance of understanding the factors that contribute to obesity and IR and implementing strategies to prevent their development.

*Effect of Change in Weight*

Several studies have shown that changes in weight are related to changes in IR, or its inverse, insulin sensitivity. Research has shown that weight gain is related to increased insulin and glucose levels (Lazzer, Vermorel, et al., 2005) and thus decreased insulin sensitivity (Reinehr et al., 2004). Similarly, several studies have reported that weight loss is
associated reduced IR or improved insulin sensitivity (Lazzer, Vermorel, et al., 2005; Reinehr et al., 2004; Steinberger and Daniels, 2003; Sudi et al., 2001). However, the amount of weight lost appears to be an important determinant of improved insulin sensitivity. In a study of 57 obese youth involved in a weight-loss intervention involving dietary and exercise programs, significant improvements in insulin sensitivity were only found in participants whose BMI standard deviation score (SDS) decreased by $\geq 0.5$ (Reinehr, Kiess, et al., 2004). In these participants, the insulin sensitivity index as measured by HOMA improved from 0.46 to 0.77 and when measured by QUICKI from 0.34 to 0.37 ($p<0.01$). When all participants were analyzed, the relationship between change in SDS BMI and HOMA-IR was moderate in strength ($r=0.39$, $p=0.001$) (Reinehr et al., 2004).

A nine-month weight loss program elicited significant reductions in insulin among obese adolescents (62% in boys, 34% in girls) (Lazzer, Vermorel, et al., 2005). This program involved both dietary education and exercise prescriptions as well. Interestingly, in participants who maintained their weight four months after the completion of the intervention, insulin levels were 50% greater than at the end of the program (month 9). In participants who regained weight at post-testing, insulin levels were only 40% greater than month 9 (Lazzer, Vermorel, et al., 2005). This may be attributable to the high BMI of these participants, even after weight loss (mean BMI = 25 for boys and 27.5 for girls). It also suggests that factors other than body mass influence fasting insulin levels in adolescents (Lazzer, Vermorel, et al., 2005). Thus, in general, weight gain is associated with increased IR, and weight loss is associated with decreased IR.
Leptin

*Function*

Leptin is a hormone released from adipose tissue (Friedman and Halaas, 1998; Zhang et al., 1994). It was discovered in 1994 and is involved in the control of food intake, energy production and weight balance. Leptin has anti-obesity effects as it decreases hunger and food consumption and increases energy expenditure, fatty acid oxidation and lipolysis (Friedman and Halaas, 1998; Fruhbeck, 2006; Havel, 2000; Havel, 2004; Korner et al., 2005; Rosenberg et al., 2005; Schwartz, 2001; Unger et al., 2004). The decreased hunger and food intake are accomplished via inhibition of neuropeptide Y (NPY) synthesis and agouti-related peptide (AgRP) and inactivation of AMP-kinase (Douyon and Schteingart, 2002; Freidman and Halaas, 1998; Porte et al., 2005; Rajala and Scherer, 2003; Unger et al., 2004). Similarly, leptin increases the activity of α melanocyte-stimulating hormone (α-MSH), which mediates satiety (Friedman and Halaas, 1998; Schwartz, 2001). Furthermore, leptin increases glucose uptake (Dyck, 2005; Ronti et al., 2006) and beta oxidation, (Dyck, 2005; Dyck et al., 2006; Ronti et al., 2006) thereby enhancing insulin sensitivity.

Leptin levels are influenced by sex, age, pubertal status, body fat, and time of day. Research has shown that females have higher leptin levels than males, regardless of weight status (Falorni et al., 1997). These sex differences become more pronounced as individuals go through puberty, with females continuing to increase and males staying stable or declining (Ahmed et al., 1999; Ankarberg-Lindgren et al., 2001; Falorni et al., 1997). One investigation found that by pubertal stages 4 and 5, boys showed marked reductions in leptin, compared to females who were continuing to increase (Falorini et al., 1997). Leptin levels are released in a pulsatile fashion and exhibit a diurnal rhythm, with peak occurring after
midnight (Ankarberg-Lindgren et al., 2001; Licinio et al., 1997; Schoeller et al., 1997; Sinha et al., 1996). Some authors have attributed this pattern of leptin release to the time of meal consumption (Schoeller et al., 1997). Leptin also exhibits a relatively small degree of day to day variability within individuals. In a sample of healthy young adults (mean age 22.3 years), leptin’s coefficient of variation was 19%, and the within subject variance accounted for 9% of the total variance (Widjaja et al., 2000).

Effect of Obesity

Leptin plays a major role in the maintenance of adipose tissue (Friedman and Halaas, 1998). Measures of adiposity are positively correlated with leptin levels such that leptin is higher in an obese individual compared to someone of normal weight (Ahmed et al., 1999; Aygun et al., 2005; Berneis et al., 1996; Falorni et al., 1997; Friedman and Halaas, 1998; Gomez et al., 2003; Gutin et al., 1999; Huang et al., 2004; Ikezaki et al., 2002; Larmore et al., 2002; Pilcova et al., 2003; Reiterer et al., 1999; Rubin et al., 2005; Sudi et al., 2000; Sudi et al., 2001; Unger et al., 2004). The increased leptin induces a state of negative energy balance through reduced food intake and increased energy burning in both human and rodent models (Friedman and Halaas, 1998). However, as fat mass and leptin levels increase with obesity, the body can become insensitive to its actions and leptin resistance often develops (Friedman and Halaas, 1998). Leptin resistance requires a greater amount of leptin to maintain normal resting energy expenditure (Lustig et al., 2004). Typical values of leptin in obese youth are ~19.9 to 24 ng/mL (Aygun et al., 2005; Coutant et al., 1998; Sudi et al., 2000), whereas levels range from ~3.8 to 7.9 ng/mL for normal weight youth (Aygun et al., 2005; Coutant et al., 1998).
Research has also shown significant associations between leptin and fasting insulin in obese youth \((r=0.32, \text{Ikezaki et al., 2002})\) \((r=0.44, \text{Rubin et al., 2005})\) \((r=0.46, \text{Gutin et al., 1999})\). Similarly, a study of obese adolescents reported positive associations among leptin and HOMA-IR \((r^2=0.56, p<0.05)\) \((\text{Huang et al., 2004})\). Perhaps the most important finding from their study was the strong relationship between leptin and HOMA-IR, even after accounting for sex, waist circumference, fat mass, pubertal stage and triglyceride levels \((R^2 = 0.43, p<0.05)\) \((\text{Huang et al., 2004})\). Sudi et al. \(2000\) studied interrelationships among hormones in obese children. In their multiple regression analysis of leptin, BMI was the only significant contributor \((R^2 = 0.28, p<0.001)\).

**Effect of Change in Weight**

Numerous studies have reported that weight loss is associated with decreased levels of leptin, signaling the body’s need for energy \((\text{Falorni et al., 1997}; \text{Gallistl et al., 2001}; \text{Geldszus et al., 1996}; \text{Lazzer, Vermorel, et al., 2005}; \text{Pilcova et al., 2003}; \text{Reiterer et al., 1999}; \text{Reinehr et al., 2005}; \text{Sudi et al., 2001})\). In each of these studies, weight loss was accomplished via alterations in diet and physical activity. One study of obese boys and girls undergoing a three-week weight loss program reported moderate to strong correlations among changes in fat mass and leptin \((r=0.43-0.71, p<0.05)\) \((\text{Reiterer et al., 1999})\). In obese girls, changes in total body mass were also related to changes in leptin \((r=0.53-0.74, p<0.05)\) \((\text{Reiterer et al., 1999})\). For boys, change in leptin was related to change in insulin \((r=0.37, p<0.05)\). A similar investigation utilizing a three-week weight reduction intervention also found strong associations among changes in weight and leptin \((r=0.45\text{ for girls, } r=0.81\text{ for boys, } p<0.01)\) \((\text{Sudi et al., 2001})\). Other researchers have reported reductions of 5% and 73% in BMI and leptin levels, respectively, following a three-week weight loss intervention.
(Gallistl et al., 2001). These studies suggest that meaningful changes in leptin and insulin can occur in a relatively short period of time.

A study of obese adolescents (mean age of 12.7 years) also found that leptin levels declined following a five-week weight loss program (Pilcova et al., 2003). In their investigation, both girls and boys lost an average of 11% of their body mass and ~3 BMI points, while leptin levels declined by 35% for girls and 58% for boys (p<0.0001) (Pilcova et al., 2003). Another study found that after weight loss, leptin levels declined and the REE:leptin ratio increased, suggesting that the body’s sensitivity to leptin increased (Lustig et al., 2004).

A study by Lazzer, Vermorel, et al. (2005) followed obese adolescents thorough a 9-month weight loss program, and at four months after completing the intervention. From month zero to month nine, leptin levels decreased 82% in boys and 60% in girls (p<0.001). At follow-up testing (month 13), leptin levels increased only slightly (7%) for those who maintained their weight, but increased markedly (74%) for those who regained weight (interaction, p=0.004). This provides further support for the close relationship between leptin and body mass.

Reineher, Kratzsch et al. (2005) studied the relationships among weight status, leptin and IR in children who lost or maintained weight. These researchers compared leptin and IR in obese children before and after a one-year weight loss program to a group of lean children matched for age, sex and pubertal status. At the start of the study, leptin levels in the obese children were 3.5 times greater than in lean children (22.0 vs. 6.3 ng/mL). At the end of the study, changes in leptin were related to: change in BMI SDS (r=0.28), change in percent body fat (r=0.44), change in IR (r=0.42) and change in insulin (r=0.43) (p<0.05) (Reinehr et
al., 2005). In the regression model for change in leptin, change in HOMA was the only significant predictor after controlling for age, sex and weight ($R^2 = 0.32$, $p=0.019$). A subgroup analysis compared 11 children with and without substantial weight loss, defined as change in SDS-BMI $\geq 0.5$ or $<0.5$ at the end of one year, respectively. In the group with substantial weight loss, significant declines in HOMA-IR were seen (2.68 to 1.85), while IR actually increased in the children who did not lose weight (2.61 to 3.76) ($p<0.05$) (Reinehr et al., 2005). Leptin levels declined slightly, but not significantly, in the group who lost weight (17.2 to 16.7) and increased in the group who did not lose weight (17.6 to 21.5, $p<0.05$). These changes were attributed to age and pubertal related increases in leptin over the one-year study period.

Researchers have also studied the influence of weight gain on leptin. In a 30-month study of 7 to 18 year old youth, strong correlations were found among leptin and BMI ($r=0.74$ for boys, $r=0.63$ for girls, $p<0.002$) (Savoye et al., 2002). In girls, leptin accounted for 18% of the variance in BMI at post-testing and a positive relationship was found between baseline leptin and subsequent increases in BMI z-score (Savoye et al., 2002). This suggests that elevated leptin levels may be predictive of future BMI gains in girls.

**Pathway to Insulin Resistance**

Leptin contributes to IR via four pathways: decreased beta-oxidation, inhibition of IRS-1, reduced JAK-2 activity, and increased nuclear factor kappa B (NF-κB) activity. Normally, leptin activates AMP-kinase which acts as an energy sensor and causes catabolic effects in the cell (Hardie et al., 2006; Havel, 2004; Rajala and Scherer, 2003; Vettor et al., 2005). The pathway begins with inactivation of acetyl coenzyme A carboxylase (ACC), thus blocking the effects of ACC in muscle cells (Minokoshi et al., 2002; Winder et al., 2001).
This leads to a reduction in malonyl CoA’s inhibitory effect on carnitine palmitoyl transferase (CPT) and consequently the activity of CPT and the rate of beta-oxidation are increased (Havel, 2004; Minokoshi et al., 2002; Vettor et al., 2005; Winder et al., 2001). This process reduces triglycerides levels in the cell and causes GLUT-4 transporter activity to increase, thereby increasing the uptake of glucose into muscle cells (Dyck, 2005; Dyck et al., 2006; Havel, 2004; Minokoshi et al., 2002; Vettor et al., 2005; Winder et al., 2001). As a result, leptin enhances insulin’s ability to lower blood glucose. However, in an obese individual, leptin levels rise in association with fat mass and eventually leptin resistance can develop (Dyck, 2005, Dyck et al., 2006; Sundell, 2005; Vettor et al., 2005; Wisse, 2004). In a leptin resistant individual, AMP-kinase is not activated and ACC activity increases, which increases malonyl CoA’s inhibition of CPT. Finally, the activity of CPT and beta-oxidation are decreased, triglycerides levels increase and GLUT-4 transporter activity and the related glucose uptake declines. Insulin secretion increases in an attempt to lower blood glucose and eventually insulin resistance develops (Dyck, 2005; Dyck et al., 2006).

Leptin has also been shown to inhibit IRS-1 in mice (Hennige et al., 2006). In this pathway, leptin increases the phosphorylation of serine (Ser-318), which decreases the attraction between IRS-1 and each insulin receptor. This causes the intracellular cascade including tyrosine phosphorylation and GLUT transporter movement to decrease and glucose uptake declines (Hennige et al., 2006).

The third mechanism by which leptin contributes to IR involves the JAK/STAT (Janus kinase / signal transducers and activators of transcription) pathway. The binding of leptin to its membrane receptor activates the JAK/STAT pathway, which begins a series of intracellular events (Fruhbeck, 2006; Porte et al., 2005). One effect of this pathway is to
activate IRS-1 and 2, thus beginning the insulin-signaling pathway within the cell (Fruhbeck, 2006; Porte et al., 2005). In a leptin resistant individual, however, JAK-2 is inhibited by SOCs3 proteins, which decreases the leptin-induced insulin signaling (Fruhbeck, 2006; Porte et al., 2005).

A fourth pathway linking leptin to insulin resistance involves the transcription factor nuclear factor kappa B (NF-κB). In this pathway, suppression of NF-κB decreased the release of proinflammatory cytokines such as TNF-α, IL-6, and IL-8, from fat and muscle tissues (Lappas et al., 2005). Interestingly, this inhibition did not affect the release of leptin, adiponectin or resistin. Reductions in the proinflammatory cytokines are related to improved beta cell function and insulin sensitivity. When the NF-κB pathway is increased, as expected in an obese individual, the release of proinflammatory cytokine increases, contributing to beta cell apoptosis and subsequent IR.

Taken together, these longitudinal studies show that loss of fat or total body mass is associated with reductions in leptin and IR in youth. These results highlight leptin’s association with IR as well as its malleability in response to changes in fat or total body mass.

**Cortisol**

*Function*

Cortisol is a hormone controlled by the hypothalamic pituitary adrenal (HPA) axis. It is classified as a glucocorticoid and is released from the adrenal cortex in response to stress and fasting or hunger. It promotes food intake via inhibiting CRH and melanocortin release and stimulating NPY (Bjorntorp, 2001; Douyon and Schteingart, 2002; Wauters et al., 2000).
Cortisol is converted into its active form via the enzyme 11β-HSD1 (11β-hydroxysteroid dehydrogenase type 1) in the liver. Cortisol increases the rate of gluconeogenesis and glycogenolysis, decreases the activity of the GLUT-4 transporter and related glucose uptake and can inhibit insulin secretion (Andrews and Walker, 1999; Lee et al., 1999). Cortisol also increases lipolysis and decreases the activity of LPL, thus slowing the breakdown of circulating triglycerides (Andrews and Walker, 1999; Lee et al., 1999). Therefore cortisol’s actions oppose those of insulin and it is often described as a “diabetogenic” hormone since it contributes to insulin resistance (Andrews and Walker, 1999). Research has shown that cortisol is released in a circadian pattern (Hermida et al., 1999; Rich et al., 1992), with significantly higher peaks occurring in the morning compared to the evening and night (Hermida et al., 1999). Finally, similar cortisol values have been reported among youth who were tested three times in a five-day period, suggesting that this hormone is somewhat stable (Rich et al., 1992). Similarly, significant intraclass correlations were found between cortisol measures taken on three consecutive days in adults (ICC = 0.886) (p<0.01) (Hackney and Zack, 2006). The coefficients of variation ranged from 12.8 to 15.7%, when comparing the three days (Hackney and Zack, 2006).

**Effect of Obesity**

Numerous studies have shown that obesity is associated with increased cortisol levels (Andrews and Walker, 1999; Bjoertorp, 2001; Douyon and Schteingart, 2002; Girod and Brotman, 2003; Sudi et al, 2000). This suggests that the activity of the HPA axis may be altered in obese individuals (Douyon and Schteingart, 2002; Reynolds and Walker, 2003). Similarly, the greater absolute levels of cortisol in the obese are attributable to their greater lean body mass compared to normal weight individuals (Strain et al., 1980). Researchers
have reported resting cortisol values in the range of 9.1 to 16.4 µg/dL for obese youth and 9.8 to 12.6 µg/dL for normal weight youth (Coutant et al., 1998; Eliakim et al., 2006). The elevations in cortisol are thought to influence NPY and therefore increase food intake (Bjorntorp, 2001). In turn, the effects of leptin are reduced, which contribute to leptin resistance associated with obesity (Bjorntorp, 2001).

However, other researchers have found that cortisol levels in obese participants are similar to or less than those in normal weight individuals. This has been attributed to increased production and secretion and faster degradation of cortisol in obese individuals (Bjorntorp and Rosmond, 2000; Douyon and Schteingart, 2002; Ghizzoni and Mastorakos, 2003; Reynolds and Walker, 2003; Wauters et al., 2000).

Effect of Change in Weight

Only one study was found that examined the effects of change in weight on cortisol and its relation to IR in children. This study had cross-sectional and longitudinal components as it compared obese youth with and without IR to normal weight youth (median age = 12) (Reinehr and Andler, 2004a). Results showed the obese participants with IR had higher mean cortisol values than the other two groups (p=0.006). Also, among the children in the two obese categories, those with IR were more obese than those without IR (p<0.03). The regression model for IR included BMI, age and cortisol ($R^2 = 0.53$, p<0.05). In the longitudinal component of this investigation, researchers compared obese children who lost weight ($\geq 0.5$ standard deviation score for BMI) at the end of a one-year intervention to those who did not ($<0.5$ SDS for BMI) (Reinehr and Andler, 2004a). This analysis revealed that cortisol and IR declined significantly only in participants who lost weight (p<0.003) (Reinehr and Andler, 2004a). In these participants, median cortisol levels declined 35% from 15.3 to
10.0 μg/dL while median HOMA scores dropped 46% from 6.3 to 3.4 (Reinehr and Andler, 2004a). These results show that in obese youth with IR, moderate weight loss of 0.8 SDS BMI influenced their HOMA score so that they were no longer insulin resistant (HOMA <4).

**Pathway to Insulin Resistance**

Cortisol has been shown to contribute to insulin resistance by via several mechanisms. Cortisol decreases the translocation of GLUT-4 transporters and associated glucose uptake (Andrews and Walker, 1999). As a result, more glucose remains in the blood and insulin levels rise in an attempt to increase the cellular uptake of glucose. Cortisol has also been shown to inhibit the release of insulin from the beta cells of the pancreas in mice (Delaunay et al., 1997). Finally, cortisol facilitates insulin resistance by increasing the production of glucose and accumulation of lipids in the cells. The increased glucose results from cortisol-stimulated gluconeogenesis, and increased activity of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphate (Reynolds and Walker, 2003). With regard to fat metabolism, cortisol increases lipolysis and decreases LPL activity, both of which increase free fatty acid levels within the cell (Andrews and Walker, 1999; Reynolds and Walker, 2003).

**Human Growth Hormone**

**Function**

Human growth hormone (hGH) is secreted from the anterior pituitary gland in response to growth hormone releasing hormone (GHRH), ghrelin, exercise and hypoglycemia. Its release is inhibited by glucocorticoids, somatostatin and insulin. Its metabolic functions include increasing lipolysis, glucose synthesis, glycogenolysis, and
decreasing glucose uptake by the liver (Clemmons, 2004; Davidson, 1987; Dieguez et al., 2000; Holt et al., 2003; Kophcick et al., 1999; Lee et al., 1999). Thus, it often considered to be an insulin-antagonist (Holt et al., 2003). Growth hormone also contributes to the maintenance and function of pancreatic islets in mice, suggesting that it is involved in insulin release and sensitivity (Liu et al., 2004). The effects of hGH are mediated by insulin-like growth factor-1 (IGF-1) and they work synergistically to increase muscle mass. The metabolic actions of IGF-1 are similar to those of insulin including decreased glucose production, increased glucose utilization, and decreased lipolysis (Holt et al., 2003).

Human growth hormone is released in a pulsatile manner (Argente et al., 1997; Bertherat et al., 1995; Rasmussen et al., 1995), with the greatest release occurring at night, as during sleep (Hermida et al., 1996; Hermida et al., 1999). Resting levels of hGH are higher in children compared to adults as children are growing and accumulating mass. Reported mean values of hGH in obese and normal weight children are 11.6 ng/mL and 14.9 ng/mL, respectively (Coutant et al., 1998).

Effect of Obesity

Numerous studies have found that obesity is associated with decreased hGH (Argente et al., 1997; Casanueva et al., 1998; Coutant et al., 1998; Dieguez et al., 2000; Douyon and Schteingart, 2002; Ghizzoni and Mastorakos, 2003; Girod and Brotman, 2003; Kamel et al., 2000; Lee et al., 1999; Nam et al., 1997; Rasmussen et al., 1995; Rasmussen et al., 2006; Roemmich et al., 2005; Saitoh et al., 1998). The reduction in hGH in obese individuals has been linked to several mechanisms. Some studies show a direct inhibition of hGH release from the pituitary gland as a result of obesity (Girod and Brotman, 2003; Kamel et al., 2000; Lee et al., 1999; Nam et al., 1997; Saitoh et al., 1998) or increased levels of FFA associated
with obesity suppress hGH release through a negative feedback loop (Casanueva et al., 1998; Girod and Brotman, 2003). Reduced hGH in the obese may also be due to elevations in leptin and cortisol (Casanueva et al., 1998; Coutant et al., 1998). High levels of leptin are thought to influence NPY and GHRH release from the hypothalamus, thus inhibiting the release of hGH (Casanueva et al., 1998; Coutant et al., 1998). The mechanisms by which high cortisol contributes to hGH suppression is not well understood, however (Casanueva et al., 1998).

Effect of Change in Weight

While few researchers have studied the effect of change in weight status on hGH in children, in general, weight loss has been associated with increased hGH concentrations. Argente et al. (1997) found that resting hGH levels increased in prepubertal children after 6 and 12 months in a weight loss program. In this study, hGH levels were similar to those in normal weight children once the obese reduced their BMI standard deviation score by 25%. Similarly, prior to weight loss, obese children had fewer daily bursts of hGH (P<0.001). These alterations in GH pulsatility have also been reported in young adults (mean ages 26.1 to 29.9) before and after weight loss (Rasmussen et al., 1995).

Pathway to Insulin Resistance

Increases in hGH have been associated with insulin resistance or reduced insulin sensitivity (Barbour et al., 2005; Clemmons, 2004; Dominici and Turyn, 2002; Dominici et al., 2005; Jeffcoate, 2002; Johansen et al., 2005; Jorgensen et al., 2005; Rizza et al., 1982; Takano et al., 2001; Weaver et al., 1995). Human growth hormone contributes to insulin resistance through several intracellular pathways. The binding of hGH to its receptor activates the intracellular JAK2 protein kinase pathway (Dominici et al., 2005; Johansen et
al., 2005; Kophcick et al., 1999). JAK2 phosphorylates and activates the insulin receptor substrate (IRS), which in turn activates the PI3K and the Akt cascade. This cascade causes the translocation of GLUT-4 to the cell membrane, which increases the uptake of glucose (Dominici et al., 2005; Johansen et al., 2005; Kophcick et al., 1999; Whiteman et al., 2002). However, SOCs proteins inhibit JAK2 and IRS, thus impairing glucose uptake (Dominici et al., 2005; Johansen et al., 2005; Mooney et al., 2001). Similarly, the intracellular pathway between PI3-k and Akt may be disrupted in the presence of hGH (Takano et al., 2001). Insulin levels increase in an attempt to lower blood glucose and eventually insulin resistance may develop.

INTERACTIONS AMONG THE HORMONES AND WITH INSULIN

The interrelationships among leptin, cortisol, hGH and insulin release are complex, as depicted in Figures 3, 4 and 5. Figure 3 depicts the relationships between the hormones and insulin release; leptin and cortisol inhibit the release of insulin, while growth hormone stimulates insulin release. Figure 4 highlights insulin’s stimulation of leptin release and its inhibition of cortisol and hGH release. Finally, Figure 5 displays how these hormones are related to one another and to insulin. The relationships depicted in the figures are discussed in each respective hormone section below. Much of what is known about these relationships is from investigations using animal models or isolated human cells. As a result, they may not be identical to in vivo events in humans. Additionally, conflicting findings have been reported for some relationships, such as hGH’s affect on leptin release, while other relationships are not well understood such hGH’s affect on cortisol release. The following section reviews how each hormone affects the release of the other three hormones.
Figure 3. The Effects of Leptin, Cortisol, hGH on Insulin Release.

Legend:  + = stimulation;  − = inhibition

Figure 4. The Effects of Insulin on the Release of Leptin, Cortisol and hGH.

Legend:  + = stimulation;  − = inhibition
Figure 5. Interrelationships Among Leptin, Cortisol, hGH and Insulin Release.

Legend: + = stimulation; − = inhibition

Insulin

Many studies have found that insulin stimulates the release of leptin (Barr et al., 1997; Boden et al., 1997; Havel, 2000; Havel, 2004; Kieffer and Habener, 2000; Lee et al., 2001; Wabitsch et al., 1996; Wauters et al., 2000). A group of researchers proposed that an “adipo-insular axis” explained this stimulatory effect of insulin on leptin release from adipose tissue (Fehmann et al., 1997). In this axis, insulin released by the pancreas promotes glucose uptake into adipose tissue, which in turn releases leptin as a signal that its energy stores are plentiful. As a result, leptin feeds back to inhibit insulin release. Other authors have supported this theoretical model as well (Havel, 2000; Havel, 2004; Kieffer and Habener, 2000; Seufert et al., 1999; Seufert, 2004; Wauters et al., 2000).

An investigation by Boden et al. (1997) studied the effects of elevated insulin levels on leptin release using a hyperinsulinemic-euglycemic clamp protocol in men (mean age 39.8 years). They reported dose dependent rises ($r=0.70$, $p<0.001$) in leptin accompanying the
increases in insulin, demonstrating the tight coupling of these hormones. Leptin levels were not affected when insulin increased by <200 pM, however, larger increases in insulin (~800 pM) were associated with substantial increases in leptin (70%) over a three day period (Boden et al., 1997). In an investigation using isolated adipose cells from humans, researchers found that leptin increased (~55%) after the addition of insulin, most notably at the higher concentrations of insulin (10^3-10^5 pmol/L) (Wabitsch et al., 1996). The effects of insulin on leptin release were amplified when cortisol was added, as discussed below. In incubated rat adipose cells, the infusion of insulin increased leptin secretion and total leptin to ~330% and ~350% of baseline values, respectively (Barr et al., 1997). Similarly, researchers found a dose-response relationship between insulin injection (25, 50, 75, 100 and 200 nM) and leptin release (ranging from 10 to 25 µg/mg) in adipose tissue of mice (Lee et al., 2001).

Research of the effect of insulin on cortisol and hGH release is scarce, however. In one of the few studies in this area, insulin was shown to inhibit 11β-HSD1 mRNA, which in turn, decreases the production and release of cortisol (Jamieson et al., 1995). The actions of cortisol and hGH oppose those of insulin, since they increase energy production via gluconeogenesis, glycogenolysis and lipolysis. As a result, resting levels of these hormones may be inversely related (Delaunay et al., 1997; Magri et al., 2001; Rubin et al., 2005).

**Leptin**

Several studies have shown that leptin inhibits the release of insulin (Fehmann et al., 1997; Havel, 2004; Kieffer and Habener, 2000; Seufert and et al., 1999; Seufert, 2004; Wauters et al., 2000). Two groups of researchers studied these relationships by infusing differing amounts of glucose into isolated human pancreatic cells and marked reductions in
insulin secretion due to leptin were found (Fehmann et al., 1997; Seufert et al., 1999). One group reported a (~15-30%) decrease in insulin secretion, only in response to the highest glucose infusion of 20mM (Fehmann et al., 1997), while a similar investigation found a 20% reduction in insulin release in response to leptin after infusion of 5.6 mM glucose (Seufert et al., 1999). The “adipo-insular axis”, as discussed above, may regulate these responses (Fehmann et al., 1997). In this axis, insulin increases glucose uptake and leptin release by adipose tissue, and in turn, leptin inhibits further insulin release (Fehmann et al., 1997). Leptin also signals a reduction in appetite, food intake and autonomic nervous system activity, further contributing to reductions in insulin production (Kieffer and Habener, 2000).

Similarly, leptin has been shown to inhibit cortisol release (Bornstein et al., 1997; Cavagnini et al., 2000; Leal-Cerro et al., 2001; Pralong et al., 1998, Wauters et al., 2000). Leptin’s inhibition of cortisol makes sense physiologically since leptin is a signal of plentiful energy stores and cortisol increases available energy. Leptin has been shown to inhibit cortisol by decreasing the activity of the hypothalamic-pituitary-adrenal (HPA) axis (Leal-Cerro et al., 2001; Wauters et al., 2000). In one study on this topic, researchers treated human adrenal cells with leptin and incubated them for 6 and 24 hours; after stimulation by ACTH, cortisol secretion was measured (Pralong et al., 1998). In the cells incubated for 24 hours, cortisol levels were only 71-75% of the control values when leptin concentrations of $10^{-7}$ M and $10^{-8}$ M were used, respectively, p<0.05 (Pralong et al., 1998). Similarly, researchers found a dose-response relationship between leptin and reductions in basal and ACTH-stimulated cortisol release in humans (Bornstein et al., 1997).

Finally, research suggests that leptin inhibits hGH release (Carro et al., 1997; Coutant et al., 1998). Possible mechanisms by which leptin inhibits GH release are through it’s
effects on GHRH and NPY at the hypothalamus or direct inhibition of hGH release from the anterior pituitary (Casanueva et al., 1998; Coutant et al., 1998; Dieguez et al., 2000; Ghizzoni and Mastorakos, 2003; Lee et al., 1999). In a study of lean and obese prepubertal children (mean ages ~9 and 10 years), 60 µg/m² of GHRH was injected to stimulate GH release (Coutant et al., 1998). Inverse relationships were found between leptin and peak GH release in lean and obese children (r=-0.51, r=-0.55, respectively, p=0.0001). These relationships were stronger than those found for insulin or body fat. In a study using rats, injection of 10 µg of leptin antiserum resulted in reductions in GH secretion when measured 6 hours after injection, compared to control rats (AUC 168 ng/mL vs. 813 ng/mL, p<0.01) (Carro et al., 1997). These studies demonstrate leptin’s role in a negative feedback loop to down regulate GH release from the anterior pituitary.

**Cortisol**

Numerous investigations have shown that cortisol stimulates leptin release (Askari et al., 2000; Berneis et al., 1996; Ghizzoni and Mastorakos, 2003; Leal-Cerro et al., 2001; Nishiyama et al., 2000; Vettor et al., 2005; Wabitsch et al., 1996; Wauters et al., 2000). A study of healthy adults (mean age ~37 years) found that infusion of the glucocorticoid hydrocortisone was related to increased leptin levels, after a lag of approximately eight hours (Askari et al., 2000). Leptin increased from ~16 ng/mL to 30 and 42 ng/mL after 16 and 20 hours, respectively. Hydrocortisone infusion elicited a 163% increase in leptin, compared to an 83% increase when insulin was infused (Askari et al., 2000). Similar results were found in a one-week study using glucocorticoid and hGH administration in adult men (Berneis et al., 1996). Leptin increased from ~2.4 ng/mL in the glucocorticoid group and ~2.0 in the glucocorticoid + hGH group to ~2.9 ng/mL by day seven (p<0.02). Finally, a study of
women (mean age 28 years) reported that the presence of cortisol and insulin increased leptin production in adipocytes by 300% (Wabitsch et al., 1996). In isolated adipocytes, the removal of cortisol led to an 80% reduction in leptin and leptin levels returned to 90% of their starting values 24 hours after cortisol was re-introduced to the cells (Wabitsch et al., 1996). Taken together, these data demonstrate cortisol’s stimulatory effect on leptin release.

Cortisol has been shown to inhibit hGH release (Barbarino et al., 1990; Ghizzoni et al., 1996). Ghizzoni et al. (1996) studied the effects of corticotrophin releasing hormone (CRH) and growth hormone releasing hormone (GHRH) on hGH secretion in a sample of children (mean age 10 years). They found a dose-dependent response in hGH, such that the smallest increases in hGH occurred when the largest dosage of CRH (2 µg/kg) was used, p<0.05. Similarly, the addition of CRH to GHRH resulted in an AUC that was ~3 times smaller than when only GHRH was administered (1,022 vs. ~3,109 µg/L over 24 hours, p<0.05). Similar results have been reported in adults (Barbarino et al., 1990).

Cortisol also inhibits the release of insulin (Delaunay et al., 1997; Lambillotte et al., 1997; Ling et al., 1998). To study the effects of cortisol on insulin release, researchers bred mice with increased glucocorticoid receptors and therefore enhanced sensitivity to glucocorticoids (Delaunay et al., 1997). These transgenic mice and a group of control mice underwent glucose tolerance tests to study their insulin response. The transgenic mice had significantly lower insulin values in response to the glucose test (7.2 vs. 9.3 µU/mL at minute 0 and 9.7 vs. 21.0 µU/mL at minute 5). Similar results were found by researchers studying the effects of the glucocorticoid dexamethasone on insulin secretion in mice (Lambillotte et al., 1997). In this investigation, they found lower insulin secretion and a slower rate of secretion in mice exposed to dexamethasone compared to controls and these differences
increased as time spent in culture increased (Lambillotte et al., 1997). These data
demonstrate the role of glucocorticoids in reducing insulin secretion.

**Human Growth Hormone**

Research examining the effect of hGH on leptin release is mixed. Some researchers
have found that hGH stimulates leptin release (Berneis et al., 1996; Fain and Bahouth, 2000; Ghizzoni et al., 2001). As described above, Berneis et al. (1996) reported a ~45% increase in
leptin in response to administration of hGH and glucocorticoids. However, the authors
suggested that the increased leptin may be attributable to the 600% rise in insulin in this
study. In rat adipose tissue, researchers found a 50% increase in leptin release and a 28%
increase in leptin mRNA in response to 10nmol/L of GH administration (Fain and Bahouth,
2000). At this time, the mechanisms by which hGH stimulates leptin release are not well
understood. Conversely, other authors have found that hGH inhibits leptin release (Isozaki et
al., 1999; Wauters et al., 2000). Isozaki et al. (1999) studied the effects of two doses of GH
(0.5 mg/kg/day and 1.65 mg/kg/day) on leptin mRNA in Zucker rats, bred to be obese and
resistant to insulin and leptin. Results showed that regardless of dose, GH decreased leptin
mRNA in epididymal fat but not in subcutaneous fat. Finally, researchers have also reported
that hGH has no effect on leptin secretion (Lee et al., 2001). This conclusion was made since
treatment of mouse adipocytes with GH (0.3 and 0.6 U) yielded no significant differences for
leptin mRNA, leptin protein or serum leptin compared to the untreated, control adipocytes
(Lee et al., 2001).

The effect of hGH on cortisol release is also unclear. No studies were found that
examined the direct effect of hGH administration on cortisol. Related research shows that
mean values for hGH and cortisol are strongly correlated in children (r=0.83 p<0.01)
(Martinelli et al., 1994). However, other researchers have failed to find significant relationships between hGH and cortisol in children \((r=-0.03, \ p=0.46)\) (Hermida et al., 1999).

Finally, researchers have described the role of hGH in stimulating insulin release (Kawabe and Morgan, 1983; Sirek et al., 1979). The influence of GH (1 or 10 \(\mu\)g/mL) on insulin release in response to glucose was studied in rats (Kawabe and Morgan, 1983). Insulin release increased by 39\% and 71\% in rats treated with 1 and 10 \(\mu\)g/mL of GH, respectively, compared to controls. Thus, a dose-response relationship was present. Additionally, research in dogs showed that injection of 10 mg/kg GH increased plasma insulin from \(<10\) to \(~33-58\ \mu\)U/mL in 10 minutes (Sirek et al., 1979).

**FITNESS AND INSULIN RESISTANCE**

Research on the relationship between physical fitness and insulin resistance in children has produced mixed findings. Some studies showed independent associations between these variables, another found no relationship between these variables, yet others reported that their relationship is mediated by body fat. The independent effect of fitness on fasting insulin levels was demonstrated following an 8-week aerobic exercise intervention in 11 to 14-year old children (McMurray et al., 2000). In this investigation, insulin decreased more in participants who improved their \(\text{VO}_2\text{max}\) (defined \(\geq 3\ \text{ml/kg/min}\) increase) compared to those who did not. Interestingly, among participants in the highest quartile of insulin at baseline, similar amounts of body fat were gained between participants who did and did not improve their \(\text{VO}_2\text{max}\); yet greater reductions in insulin \((p<0.05)\) were found in participants who increased their \(\text{VO}_2\text{max}\). This showed that body mass did not influence the relationship between fitness and insulin, at least for participants in the highest quartile of insulin. Another study of youth reported that \(\text{VO}_2\text{max}\) was negatively associated with fasting insulin
(r= -0.42) and HOMA-IR (r= -0.27) (p<0.001) (Allen et al., 2007). In this investigation, VO$_2$\textsubscript{max} was expressed in units of ml oxygen per kilogram lean body mass per minute, thus removing the influence of body fat, which influences insulin concentrations (McMurray et al., 2000), from the analyses. An investigation by Gutin et al. (1994) reported a correlation of -0.72 (p<0.001) between insulin and peak VO$_2$ in 7 to 11-year old children with varying amounts of body fat (10 to 58%), suggesting a moderate to strong association between these variables. Finally, after a 12-week aerobic exercise intervention in overweight children, insulin sensitivity and fitness (PWC$_{170}$) increased by 23% and 24%, respectively (Nassis et al., 2005). In their investigation, the improved insulin sensitivity was evident by a decrease in insulin AUC (area under the curve) in response to an oral glucose tolerance test; however, no changes in HOMA-IR or fasting insulin were found.

Several studies have suggested that the relationship between fitness and IR is mediated by fat mass (Ball et al., 2004; Ferguson et al., 1999; Lee, Bacha et al., 2006; Ruiz et al., 2007; Slinger et al., 2007). In a study of 8 to 17-year old children, cardiorespiratory fitness (CRF), expressed as VO$_2$\textsubscript{peak} (ml/kg/min) was associated with insulin sensitivity, however, after controlling for fat mass, CRF was not a significant predictor of insulin sensitivity (p>0.05) (Lee, Bacha et al., 2006). Similarly, in a large study of 9 to 10-year old children, participants were divided into quartiles of fitness based upon their VO$_2$\textsubscript{max} (ml/kg/min), from a cycle ergometer test (Ruiz et al., 2007). Fasting insulin was significantly higher in participants in the lowest quartiles of fitness, compared to the upper quartiles, while glucose did not differ among quartiles (Ruiz et al., 2007). This suggests that insulin sensitivity was greatest in the upper quartiles of fitness. Although not directly compared to fitness or insulin, percent body fat was greatest in the lowest quartile (p<0.001) and may
therefore impact to the relationship between insulin and fitness (Ruiz et al., 2007). In another study of overweight youth, researchers reported correlations of 0.42 (p<0.05) between VO$_{2\text{max}}$ (ml/kg/min) and insulin sensitivity, however, after controlling for fat mass, gender, pubertal status and lean tissue, the association fell to r=-0.01 (p>0.05) (Ball et al., 2004). However, when studies include only obese children, a large portion of the population is omitted, which may skew the results. Similarly, researchers reported correlations of -0.192 and -0.226 between HOMA-IR and endurance time in 7 to 8-year old boys and girls, respectively (Slinger et al., 2007). After adjusting for body fat, the correlations were reduced by approximately 50% (-0.095 in boys, -0.107 in girls) (Slinger et al., 2007). It is possible at the young age of 7-8 years, insulin resistance has not yet developed.

Finally, Shaibi et al. (2006) reported no differences in fitness of children with normal vs. impaired glucose tolerance (p>0.05). These findings remained regardless of the units of VO$_{2\text{max}}$ (L/min, ml/kg/min and ml/kg\text{f bm}/min), suggesting that fitness may not play a major role in the development of IR. Taken together, evidence is accruing to show that a relationship between fitness and insulin in children exists. However, further clarification of this relationship is needed and studies of IR in children should consider the effects of fitness, along with possibly gender, pubertal status and ethnicity.

SUMMARY

This review highlighted the relationship between weight status and IR, several hormones that influence IR (leptin, cortisol and hGH), the effect of weight status change on these hormones, and finally the complex interrelationships among these hormones and with insulin. No previous investigation has simultaneously studied the relationships among changes in leptin, cortisol, hGH, and insulin using a longitudinal model of natural changes in
weight status change in youth. As a result, many questions remain to be answered. For example, will changes in IR in participants who increased weight status change be greater than those who decreased weight status? Which of the hormones has the strongest relationship with IR in youth? How do the relationships between the hormones and IR differ between the four weight status groups (NN, NO, OO, ON)? These questions, among others, are answered in the three manuscripts accompanying this literature review.
APPENDIX B
Extended Methods

Participants

Participants were obtained from the Cardiovascular Health in Children III (CHIC) study. The CHIC study was a longitudinal investigation of cardiovascular disease risk factors in youth in rural North Carolina, with data collected annually from 2000 to 2003. The present investigation selected participants from Cohort 5, during their first and third years of study (referred to as t1 and t2 in this investigation). The participants were in 3rd through 5th grade at t1 (age range of 7 to 13 years), and in 5th through 7th grade at t2 (age range of 9 to 15 years). A total of 1566 participants belong to Cohort 5, of which 120 are included in this investigation.

Participants were selected from the larger cohort and divided into four groups based upon their weight status at t1 and t2. The groups are comprised of participants who were: normal weight at t1 and overweight at t2 (NO), normal weight at both t1 and t2 (NN), overweight at t1 and normal weight at t2 (ON), and overweight at both testing times (OO). Normal weight was defined as < 85th and > 5th percentile BMI for age and sex and overweight as ≥ 85th percentile BMI for age and sex. These four groups were created in order to compare the effects of becoming overweight (NO) vs. remaining normal weight (NN), as well as to compare the effects of becoming normal weight (ON) vs. maintaining overweight status (OO). Comparisons were also made between the NO and ON groups, in order to determine the differences between positive and negative changes in weight status.

Of the 1566 participants in Cohort 5, 60 fit the criteria of increasing weight status, of which 35 had complete data. A comparison group was formed by randomly selecting 35 participants out of the 499 participants from Cohort 5 who were normal weight at both time
points (NN). The participants were matched to those in the NO group by pubertal status at t₁, sex, and race since these variables are known to influence the outcome variables in this investigation. Similarly, 35 participants fit the criteria of decreasing weight status, of which 25 had complete data. A comparison group was formed by randomly selecting 25 out of the 386 participants from Cohort 5, who were overweight at both time points (OO). Similarly, these participants were matched to those in the ON group by pubertal status at t₁, sex, and race.

**Data Collection Procedures**

All data were obtained at the participants’ schools by teams of research assistants (RAs). The RAs measured height using a stadiometer (Perspective Enterprises, Portage, MI) and body mass using an electronic scale (Model 2101KL, Healthometer Medical, Bridgewater, IL), and these measurements were taken in duplicate, to the nearest 0.1 cm and 0.1 kg, respectively. Height and body mass were then used to derive BMI (kg/m²). Percent body fat was measured using the sum of skinfolds obtained at the subscapula and triceps sites using Lange calipers (Cambridge Scientific, Cambridge, MD). The measurements were taken in triplicate using the guidelines from the NHANES III (1974). The values were subsequently used to calculate percent body fat in equations specific for sex, race and pubertal stage (Slaughter et al., 1988). The stage of pubertal development was quantified using a self-report survey specific for each sex (Petersen et al., 1988). Finally, blood draws were taken via standard venipuncture techniques between 7 and 9 am, after an overnight fast that was verified by the RAs. Blood samples were centrifuged and the plasma was separated
into storage vials. The plasma was placed on dry ice and shipped back to the University where they were frozen at -80\(^\circ\)C for later analysis.

Since multiple RAs were involved in data collection, quality control measures were in place to increase the accuracy of the measurements. All RAs were trained by the same investigator and required to meet strict criteria for reliability and precision before gathering any data. To further ensure quality control, height, body mass and skinfolds were measured by more than one RA for every tenth participant.

**Variables Measured from Blood**

Insulin, leptin, cortisol and hGH were analyzed from the stored plasma via radioimmunoassay (RIA) procedures. All samples were measured in duplicate, with the exception of three samples in the hGH assay that were run as singlets due to limited plasma samples. Insulin and leptin were measured using mobile phase, double-antibody RIA kits, with sensitivities of 2.0 \(\mu\)U/mL and 0.5 ng/mL, respectively (LINCO Research, St. Charles, MO). Cortisol was assessed using solid phase, single-antibody RIA kits, with a sensitivity of 0.2 \(\mu\)g/dL (Diagnostic Products Corporation, Los Angeles, CA). Human growth hormone was measured using mobile phase, double-antibody kits, with a sensitivity of 0.31 ng/mL (MP Biomedicals, Costa Mesa, CA). The mean coefficients of variation for the leptin, cortisol and hGH assays were 8.1\%, 6.4\%, and 7.2\%, while the intraassay coefficients of variations were: 5.8\%, 4.5\%, and 8.5\%, respectively. Finally, insulin resistance was calculated using homeostatic model assessment (HOMA-IR) using the following equation: fasting insulin concentration (\(\mu\)U/mL) \times\) fasting glucose concentration (mmol/L) / 22.5 (Matthews et al., 1985).
**Power Analyses**

Using the equation presented by Oakes and Feldman (2001), the minimal detectable difference in HOMA-IR for normal weight participants was 0.56 units, based on the following: standard deviation in HOMA of 0.95 (Atabek et al., 2007), power of 0.80, alpha of 0.05, correlation of 0.6 and a sample size of 35 group. Similarly, the minimal detectable difference in HOMA-IR for overweight participants was 1.7 units, based upon a standard deviation in HOMA of 2.5 (Allen et al., 2007; Conwell et al., 2004), power of 0.80, alpha of 0.05, correlation of HOMA scores over time of 0.6 and a sample size of 25 per group (Oakes and Feldman, 2001). Thus, there appeared to be adequate power for all analyses comparing HOMA-IR among the four weight groups. For the simple correlation analyses, the equation by Sokal and Rohlf (1995) indicated that the sample size of 120 provided sufficient power to detect a correlation of 0.25. This was based upon a power of 0.80 and an alpha of 0.05. This provided sufficient power to detect significant correlations among variables in this investigation, as those reported in similar investigations are greater than 0.25.

Minimal detectable differences were also calculated for change in leptin, cortisol and hGH. The minimal detectable difference in leptin in normal weight children was 0.24 ng/mL based upon a standard deviation 0.4 ng/mL (Coutant et al., 1998), power of 0.80, alpha of 0.05, correlation of 0.6 and a sample size of 35. For cortisol, all numbers were the same as those used for leptin expect for the standard deviation which was 1.0 µg/mL (Coutant et al., 1998). This produced a minimal detectable difference of 0.60 µg/mL in cortisol in normal weight children. Finally, the standard deviation used for hGH was 1.2 ng/mL (Coutant et al., 1998), with all other numbers identical to those for leptin and cortisol, resulting in a minimal detectable difference of 0.72 ng/mL in normal weight children.
When overweight children were considered, the minimal detectable differences in leptin, cortisol and hGH were found to be: 1.20 ng/mL, 0.57 µg/mL, and 1.06 ng/mL, respectively. These values were based upon the following standard deviations: leptin = 1.7 ng/mL, cortisol = 0.8 µg/mL and hGH = 1.5 ng/mL (Coutant et al., 1998). Power was set at 0.80, alpha at 0.05, and a conservative 0.6 correlation was used for each, with a sample size of 25 (Oakes and Feldman, 2001). Taken together, the minimal detectable differences were all smaller than the differences expected from previous research in children, and the sensitivity of the assays was small enough to detect minute changes. This suggests that we had sufficient statistical and analytical power to detect physiologic differences when they existed.

**Data Reduction**

The concentrations of insulin, leptin, cortisol and hGH obtained from the RIA techniques were examined using box plots and z-scores. When outliers were detected, we examined the value to determine if it was physiologically possible. For those values that were outside of the physiologic range, a proportional mean transformation was performed, using the mean from the respective group (i.e. NN, NO, OO or ON) and the participant’s corresponding blood sample from the other time point. There were a similar number of imputations among the four weight status groups and at each time point. More specifically, mean transformations were done in the NO group for four samples at t₁ and three samples at t₂, out of the 105 samples at each time point. For the NN group, transformations were performed for three and four samples at t₁ and t₂, respectively, out of the 105 samples at each time point. Likewise, values were transformed for two and three samples in the OO group.
and four and five samples in the ON groups at $t_1$ and $t_2$, respectively, out of the 75 samples at each time point. Altogether, 28 out of 720 blood samples, or 3.9% of the data points, were transformed, which is well below the 10% commonly permitted by statisticians.
REFERENCES


