

PHYSICAL ACTIVITY, DAILY CORTISOL PATTERNS AND THE METABOLIC
SYNDROME IN OBESE ADOLESCENTS

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ABSTRACT

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The influence of cortisol and stress in the development of obesity and metabolic syndrome has received increased interest in recent years due to the high prevalence of overweight and obesity in children, youth, and adults. Some studies suggest that physical activity may attenuate the relationship between stress and metabolic syndrome, but more studies are necessary to fully understand this relationship. Many studies have shown a relationship between stress and metabolic syndrome using survey measures, but researchers have not linked survey measures to HPA axis activity. Therefore, the purpose of this study was to examine the relationship between stress (using daily cortisol and survey measures) and metabolic syndrome in obese youth, and to examine the potential moderating effects of physical activity on that relationship. **METHODS:** Obese adolescents were recruited from the Healthy Weight Center (HWC) and Academic General Pediatrics (AGP) clinics at Helen DeVos Children's Hospital in Grand Rapids, MI. Height, weight, waist circumference, blood pressure, and pubertal stage were obtained at the time of a routine clinic visit, and BMI was calculated. Participants completed the Pediatric Symptom Checklist (PSC) to evaluate psychosocial function (stress). Physical activity (steps/day and minutes per day spent in moderate-to-vigorous physical activity – MVPA) was monitored using the SenseWear Pro III armband (SWA). Participants provided saliva samples (while at home on a weekend day) that were taken at prescribed times: 1) immediately upon

waking; 2) 30-minutes after waking; 3) 3 hours after waking; 4) 6 hours after waking; 5) 9 hours after waking; 6) 12 hours after waking. Fasting measures of HDL cholesterol, triglycerides, and glucose were obtained from physician-ordered blood draws and used with waist circumference and systolic blood pressure to calculate a continuous metabolic syndrome risk score (cMetS).

RESULTS: A total of 50 subjects (15 boys, 35 girls; mean age 14.8 ± 1.9 y) agreed to participate. The mean cMetS score was 4.16 ± 4.30 and did not differ by clinic or sex. Subjects participated in approximately 46 min of moderate-to-vigorous physical activity per day. Mean cAUC was 1.337 ± 0.867 $\mu\text{g/dl}$ (1.180 ± 0.753 $\mu\text{g/dl}$ and 1.408 ± 0.922 $\mu\text{g/dl}$ for males and females, respectively) and did not differ by sex or clinic. No significant relationship was found between cAUC and cMetS ($R^2=0.113$, $p=0.66$). Additionally, neither the interactions of steps/day nor MVPA with cAUC significantly predicted cMetS. Partial correlations revealed a significant inverse relationship between PSC total score and cAUC ($r=-0.45$, $p=0.04$). Significant inverse relationships were also found between cAUC and PSC total score, internalizing score, and externalizing score, as well as between all three PSC scores and cortisol sample 2. Finally, no significant relationship was found between PSC score and cMetS, nor was there a significant moderating effect of steps/day or MVPA on this relationship. **DISCUSSION:** The results of this study did not support a relationship between either cortisol or PSC scores and MetS in this sample, nor was there evidence of a moderating effect of physical activity. However, more research is necessary, including survey measures that assess specific domains of stress and longitudinal data to identify the timing of altered cortisol release, metabolic effects, and exposure and reactions to stressors

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CHAPTER 1: INTRODUCTION

Background and Significance

The past several decades have seen a dramatic increase in the prevalence of overweight and obesity among youth in the United States. Currently, an estimated 32% of U.S. youth 6-19 years of age are overweight or obese (1). The current epidemic of childhood obesity, along with its immediate and long-term health consequences, makes this disorder one of the most important, if not the most important, health issue facing youth today (2–6). Beyond obesity, excess intra-abdominal adipose tissue, or central adiposity, is thought to be especially problematic, given its strong links to the metabolic syndrome (MetS)(7). The MetS is defined as a clustering of cardiovascular risk factors including dyslipidemia, hypertension, insulin resistance/glucose intolerance, and abdominal obesity. Specific adult diagnostic criteria, shown in Table 2.1, have been proposed by the International Diabetes Federation (IDF)(8), World Health Organization (WHO) (9), National Cholesterol Education Program – Third Adult Treatment Panel (NCEP-ATP III)(10) and the European Group for the Study of Insulin Resistance (EGIR)(11). Several adaptations of the adult criteria have been proposed for use with children and adolescents. These definitions have been summarized and condensed by the IDF (12). One set of criteria, defined by Cook and colleagues (13), involves the adaptation of the NCEP-ATP III (see Table 2.2) for a pediatric population. The child and adolescent cut points proposed by the IDF classify children by age (12), allowing for growth-related variation in certain variables such as waist circumference. A comparison of these two classification systems is shown in Table 2.2. Publication of age- and sex-specific percentiles based on NHANES III data have made it

possible to classify children and adolescents appropriately for each factor of MetS *except* glucose tolerance/insulin resistance (14). Age- and sex-specific percentiles have not been computed for glucose because glucose levels are known to stay fairly constant throughout childhood and adolescence (14; 15). Thus, it is necessary to use different criteria for this factor. To date, the cut-points and percentiles provided by Cook's group have not been used together to classify MetS.

It is difficult to express the prevalence of MetS among US children and adolescents with great certainty, because of the numerous classification schemes available. Current estimates indicate that 4-7% of children and adolescents in the US possess MetS, and that among obese adolescents the prevalence of MetS is approximately 30% (13). However, MetS has been reported to be present in as many as 49-53% of severely obese adolescents (16; 17). Similar to prevalence rates for overweight and obesity, there are ethnic (18) and socioeconomic status (SES) (19) differences in the prevalence of MetS. Among ethnic groups, the highest prevalence is found in Mexican American youth (5.6%), followed by white (4.8%) and black (2.0%) youth. Rates are also higher among youth living below the poverty level (5.7%) as compared to those living above the poverty level (3.7%) (13). Boys tend to have a higher prevalence of MetS than girls (6.1% vs. 2.1%, respectively). Prevalence of MetS appears to be highest during puberty (7.2% during pubic hair stage 3) (13), which may correspond with the transient insulin resistance that is seen during this time (20).

It is now well established that cardiovascular risk factors cluster in childhood (21), track over time (22–26), and increase the accumulation of fatty streaks in the coronary arteries of adolescents and young adults (27–29). In fact, risk factors that cluster tend to track more strongly than each individual risk factor when examined independently (26). Pooled evidence

from the Bogalusa Heart Study and Cardiovascular Risk in Young Finns study indicates that pediatric MetS is associated with increased risk of adult MetS. Furthermore, the presence of MetS during adolescence is associated with Type II Diabetes Mellitus and high carotid intima-medial thickness in adulthood (27). A recent analysis of data pooled from large longitudinal studies indicates risk factor measurements are most predictive of adult CV risk beginning at the age of nine years (29). Data from the Bogalusa Heart study also suggest that not only do adverse levels of CVD risk factors (clustered as MetS) track into adulthood, individuals with favorable risk factor profiles in childhood are less likely to display MetS in adulthood (25). Thus, the early prevention and treatment of pediatric MetS are important areas of study.

Following an adolescent growth spurt, fat is deposited at high rates, especially around the abdomen (30; 31). This increased fat deposition combined with lifestyle changes including decreases in habitual physical activity (32) and the dramatic changes in the hormonal environment that occur during adolescence (33) make adolescence a particularly vulnerable period in the development of obesity and metabolic syndrome. Recent studies have shown that early adolescence (ages 11-13 y) maybe be the time when CVD risk is most strongly influenced by body fatness and cardiorespiratory fitness (33). Additionally, adolescents who are at high risk for CVD may reap the largest benefits from increased OA and cardiorespiratory fitness, further suggesting that adolescence is an important time to intervene in an attempt to reduce the development of MetS (34–36).

Physical activity and diet are typically considered the most important factors in the prevention and treatment of both obesity and MetS (37), however these two factors explain only a portion of the total variance in either trait (38). An often overlooked but equally important factor is the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and altered cortisol

secretion in response to stressful situations (39–41). Alterations in the normal activity of the HPA axis and disruption of the normal daily pattern of cortisol secretion can influence both glucose and fat metabolism. Some resulting changes, such as the activation of lipoprotein lipase, promote storage of lipids in visceral adipocytes (39) and thus, the accumulation of excess adipose tissue. This notion is supported by research involving individuals with Cushing's disease, in whom chronically elevated cortisol levels are associated with greatly increased visceral adipose tissue (39). Other studies in apparently healthy adults and children have examined the relationships between cortisol and obesity (42–46) including three studies from our group (47–49). To date, the results are equivocal, which may be partially explained by methodological limitations. The largest potential limitation of previous studies is the use of a single cortisol sample collected immediately upon waking or at another point in the morning. A comparison of multiple samples taken over a 12-hour period and waking samples has shown that the waking sample alone fails to predict mean cortisol levels over the course of the entire day (50). Thus, a single waking salivary cortisol sample is not likely to be the most accurate way to examine possible relationships between cortisol and obesity; in fact, the immediate waking sample may actually be more indicative of HPA axis activity during the final stages of sleep rather than the diurnal pattern (50).

Stress can also be examined using psychosocial measures that assess a number of different domains and types of stressors. Another variable of interest is psychosocial functioning, which is related to stress in the sense that optimal psychosocial functioning is necessary to adequately cope with and tolerate stressors (51). Individuals with poor psychosocial functioning may experience a number of day-to-day difficulties, including poor self esteem and a lack of supportive peer relationships. Certain psychosocial domains, such as depression, anxiety,

attention difficulties, and behavioral problems are associated with alterations in HPA axis activity (52–54). Altered cortisol responses to stress are often seen among children and adolescents who are victims of bullying or experience behavior problems (55–57). Whether these experiences are associated with higher or lower cortisol levels seems to vary based on the type of stressor or psychosocial difficulty experienced, and may also vary when comparing acute cortisol responses to stress and basal cortisol levels (55; 56; 58).

Several surveys exist to evaluate overall psychosocial function or specific domains such as experience with bullying, lifetime or recent exposure to stressful events, depression, and anxiety (59). The Pediatric Symptom Checklist is a survey often used in clinical practice to evaluate overall psychosocial functioning and identify impaired functioning in healthy children and adolescents seen in primary care, as well as individuals with chronic illnesses (60–62). When using this checklist, a higher score is indicative of poorer psychosocial functioning, which may be associated with behavior problems, depression, attention difficulties, or other psychological difficulties, as well as altered cortisol patterns (52–54; 60).

Several investigators have described the daily cortisol pattern in children (46; 54; 63). However, few have considered the daily cortisol pattern in conjunction with obesity. In adults, elevated daily cortisol levels predict increased body mass index (BMI) (64), and those with MetS (65) or increased abdominal fat (66) exhibit increased HPA axis activity as determined by serum, urinary, and salivary measures of cortisol. The relationship between HPA axis activity and MetS in adults suggests that understanding their relationship is important to better understand the etiology of obesity and MetS in youth, as well. To our knowledge, no studies have used both survey and biological measures of stress (cortisol) to evaluate the relationships between stress and MetS in adolescents. Therefore, it is difficult to compare results from studies using surveys

to those using cortisol, and more research is necessary to understand the role of each type of measure in evaluating these relationships.

As previously mentioned, physical activity is a cornerstone in the prevention and treatment of child obesity and MetS (38). However, the role of physical activity in altering the relationship between stress and MetS is largely unknown. Holmes et al. (47) previously showed that physical activity attenuates the relationship between survey-measured anxiety and MetS in boys, and another study found that post-exercise cortisol was 32% lower than pre-exercise levels in obese children (67). Therefore, it is reasonable to hypothesize that habitual physical activity would attenuate the relationship between stress (measured by surveys and/or cortisol levels) and MetS in obese children and adolescents.

The **overall purpose of this dissertation** is to examine the relationships between habitual physical activity, stress (via survey and daily cortisol levels), and MetS in obese adolescents. The **organization** of this dissertation proposal is as follows: Chapter 1 – Introduction, Chapter 2 – Review of Literature, and Chapter 3 – Methodology, Chapter 4 – Results, and Chapter 5 – Discussion.

Specific Aims and Hypotheses

Specific aim 1: Determine the relationship between daily cortisol levels and the continuous metabolic syndrome score (cMetS) in obese adolescents. It was hypothesized that cortisol area under the curve (cAUC) will be positively related to cMetS.

Specific aim 2: Determine if habitual physical activity (MVPA and/or steps/day) attenuates the relationship between daily cortisol and cMetS in obese adolescents. It was hypothesized that

there will be a significant interaction of PA and cAUC, such that PA will attenuate the relationship between cAUC and cMetS.

Specific Aim #3: Determine the relationship between psychosocial functioning score and cAUC in obese adolescents. It was hypothesized that psychosocial functioning will be positively related to cAUC.

Specific Aim #4: Determine the relationship between psychosocial functioning score and cMetS in obese adolescents. It was hypothesized that psychosocial functioning will be positively related to cMetS.

Specific aim 5: Determine if habitual physical activity (MVPA and/or steps/day) attenuates the relationship between psychosocial functioning score and cMetS in obese adolescents. It was hypothesized that there will be a significant interaction of PA and psychosocial functioning, such that PA will attenuate the relationship between psychosocial functioning and cMetS.

Significance of the Dissertation

Given the complex etiology of obesity and MetS, it is expected that the outcomes of this study will improve current understanding of the relationship between cortisol and MetS in obese adolescents. Additionally, we hope to determine the influence of regular habitual physical activity on this relationship. Because obese adolescents are at increased risk of MetS, it is important to identify and reduce factors that may additionally contribute to that risk. Excess cortisol secretion is known to have detrimental effects on glucose tolerance, insulin sensitivity, and blood pressure (39; 40). If the increased HPA axis activity is attenuated by physical activity, even in obese adolescents, a positive effect may be observed on metabolic risk factors even if

weight loss does not result. Thus, results of this study may provide important insight into the role of both physical activity and stress reduction programs for pediatric obesity.

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CHAPTER 2: REVIEW OF LITERATURE

Introduction

This review of the literature covers several areas related to this dissertation including: child obesity, metabolic syndrome, stress and the stress response, and the relationships between physical activity, stress and the metabolic syndrome (MetS). There are several excellent review papers in several of these areas [e.g., child obesity (1), physical activity and obesity (2), physical activity and MetS (or CVD risk factors) in children (3), assessment of stress (4), and recently a seminal review on physical activity, stress, and MetS (5)]; therefore, some sections will be abbreviated compared to others.

Child Obesity: Prevalence and Consequences

Prevalence. The past several decades have seen a dramatic increase in the prevalence of overweight and obesity among youth in the United States. Presently, an estimated 32% of U.S. youth are overweight or obese (6). A recent report indicated that among a diverse group of children and adolescents, 37.1% were overweight, 19.4% were obese, and 6.4% were extremely obese ($\text{BMI} \geq 1.2 \times 95\text{th percentile}$) (7).

Compared to their normal weight peers, overweight and obese youth are more likely to be overweight or obese as adults. Relative risks of adult obesity reported in the literature range from 2.0-10.0 for overweight children and increase into adolescence [RR 12.0 (male) to 15.0 (female)] (8). While most obese adults were not overweight or obese as youth, as many as 22-58% of overweight adolescents become obese adults and 24-90% of obese adolescents become obese adults (1; 8). Those youth who are obese during late adolescence seem to be especially susceptible to adult obesity (1). Fat deposition, especially on the trunk, occurs at high rates

following the adolescent growth spurt in stature (9; 10); thus, adolescence may represent an especially important opportunity for early intervention to prevent adult obesity (1).

Obesity during the childhood and adolescent years is also associated with a number of short-term health consequences. Obese youth tend to have lower self esteem than their normal weight peers, are often the victims of bullying and teasing, and are less likely to form strong friendships (11; 12). Behavior problems also tend to be more frequent among obese youth (11). In addition to these psychosocial factors, up to 70% of young girls (including those who are at a normal weight) are actively trying to lose weight or have tried in the past (12). Rates of psychological problems appear to be higher among clinically obese youth than the non-clinical population (13).

Results from the Bogalusa, Muscatine, and Pathological Determinants of Atherosclerosis in Youth (PDAY) studies have highlighted the early emergence of CVD risk factors among overweight and obese youth (14; 15). Hypertension occurs at a higher rate among obese youth than their normal weight peers and some research has shown that blood pressure in obese youth may be especially sensitive to dietary sodium intake. Obese youth also exhibit high total and low-density lipoprotein (LDL) cholesterol, high triglycerides, and low serum high-density lipoprotein (HDL) cholesterol levels (16).

Physically, obese youth are susceptible to a number of comorbidities, some of which occur as a result of the elevated CVD risk factors discussed here. Reported abnormalities of the endocrine system include menstrual irregularities (amenorrhea, oligomenorrhea, early menarche, and polycystic ovarian syndrome), insulin resistance, excess androgen production (especially in girls) (12). Obese youth are at increased risk for gall stones and liver damage ranging from the

relatively mild fatty liver disease to steatosis, steatohepatitis, or even cirrhosis, and sleep apnea (11; 12).

Long-term medical and socioeconomic consequences are also a concern. As discussed previously, obesity tends to track into adulthood. The same is true for other CVD risk factors that tend to co-occur with obesity. Results from the PDAY study indicate that deposition of plaques within the coronary arteries begins as early as 15 years of age (15). Sixty-one percent of children participating in the Bogalusa Heart Study who were in the highest quintile of CVD risk (indicated by multiple risk factor index) at baseline remained in the highest quintile 8-years later, indicating clustered risk factors persist beginning in childhood (17). The persistence of obesity and CVD risk factors from early life into adulthood increases the body's exposure to these factors beyond what was traditionally seen with adult onset of these traits. Thus, individuals who are obese as youth may experience increased morbidity and mortality (cardiovascular and all-cause) as adults. Additionally, menstrual abnormalities experienced during adolescence and young adulthood may translate to fertility problems and increased risk of gestational complications (12).

What is Metabolic Syndrome?

The term “metabolic syndrome” refers to the clustering of several risk factors for cardiovascular disease including abdominal or visceral obesity, dyslipidemia, hypertension, and insulin resistance or glucose intolerance. The presence of MetS is associated with increased risk of disease (including cardiovascular disease, diabetes, and some cancers) and all-cause mortality (18). Four definitions of adult MetS are widely used; these include definitions proposed by the World Health Organization (WHO), the International Diabetes Foundation (IDF), and National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), and the European

Group for the Study of Insulin Resistance (EGIR). These definitions have been reviewed by Huang (19) and are summarized in Table 2.1. Additionally, researchers have proposed several definitions for the diagnosis of MetS in children and adolescents, which the IDF has condensed into one, universal definition of MetS for children and adolescents (Table 2.2).

Recent data indicate that 4%-7% of children in the US possess MetS (20–22). A clear gradient exists across weight classifications, with the lowest prevalence being reported at less than 1% in normal weight youth (22) and as high as 38.7% in moderately obese and 49.7% in severely obese youth (21). Additionally, there is a clear increase in the risk of MetS with increasing obesity, such that each half-unit increase in BMI z-score increases the risk of MetS (OR = 1.55) (21). The prevalence of MetS does not appear to differ by ethnic group (22); however sex differences do exist, with boys generally exhibiting a higher prevalence than girls (~9% vs. ~4%, respectively) (22).

The prevalence of MetS in youth reported in the literature varies based on the classification system used. As a result of this variation and in response to the low prevalence of MetS among youth, several researchers have proposed the use of a continuous metabolic syndrome score for the evaluation of risk in these age groups. Scores have been derived using population-specific z-scores of the relevant components, principal component analysis, and percentile rankings. These continuous scores (cMetS) are used as an index of cardiovascular risk, indicating whether a child or adolescent is at risk for the development of MetS and cardiovascular diseases (23).

Table 2.1. Summarized adult definitions of the metabolic syndrome.

Criteria	WHO (1998)	IDF (2005)	NCEP-ATP III (2005)	EGIR (1999)
Obesity	Waist:hip ratio >0.90 (M) or >0.85 (W); or BMI >30 kg/m ²	Waist circumference ≥ 94 cm (M) or ≥ 80 cm (F)	Waist circumference > 40 in (M) or > 35 in (F)	Waist circumference ≥ 94 cm (M) or ≥ 80 cm (F)
Hyperglycemia	Insulin resistance (by IGT, IFG, T2D or other evidence)	Fasting glucose ≥100mg/dL	Fasting glucose ≥100mg/dL or pharmacologic treatment	Hyperinsulinemia (plasma insulin >75th percentile)
Dyslipidemia	TG ≥150mg/dL or HDL-C: <35mg/dL (M) or <39mg/dL (F)	TG ≥150mg/dL or pharmacologic treatment	TG ≥150mg/dL or pharmacologic treatment	TG ≥177mg/dL or HDL-C <39mg/dL
Dyslipidemia (additional)	--	HDL-C: <40mg/dL (M), <50mg/dL (F); or pharmacologic treatment	HDL-C: <40mg/dL (M), <50mg/dL (F); or pharmacologic treatment	--
Hypertension	≥140/90 mmHg	>130 mmHg systolic or >85 mmHg diastolic, or pharmacologic treatment	>130 mmHg systolic or >85 mmHg diastolic, or pharmacologic treatment	≥140/90 mmHg or pharmacologic treatment
Other	Urine albumin ≥20μg/min or albumin:creatinine ratio ≥30mg/g	--	--	--
Threshold for diagnosis	Insulin resistance or diabetes, plus 2 of the 5 criteria	Obesity, plus 2 of the 4 criteria	Any 3 of the 5 criteria	Hyperinsulinemia, plus 2 of the 4 criteria

Criteria in bold are absolutely required for a positive MetS diagnosis; IGT = impaired glucose tolerance; IFG = impaired fasting glucose; T2D = type 2 diabetes; TG = plasma triglyceride; HDL-C = high density lipoprotein cholesterol; Adapted from Huang, 2009.

Table 2.2. International Diabetes Federation (IDF) consensus definition of the metabolic syndrome for children and adolescents (age 6-16 yrs).

	Age group	Obesity (WC)	Triglycerides	HDL-C	Blood Pressure	Glucose
IDF Consensus Definition	6 - <10	≥ 90 th percentile	MetS not diagnosed. Further consideration required in cases of family history of MetS, hypertension, cardiovascular disease, dyslipidemia, and/or obesity.			
	10 - < 16 Metabolic Syndrome	≥ 90 th percentile or adult cut-off if lower	≥ 150 mg/dL	< 40 mg/dL	≥ 130 mmHg systolic or ≥ 85 mmHg	Fasting glucose ≥ 100 mg/dL (OGTT recommended for ≥ 100 mg/dL or known T2D)
	16+ Metabolic Syndrome*	≥ 94 cm (M) or ≥ 80 cm (F)	TG ≥ 150mg/dL or pharmacologic treatment	< 40 mg/dL (M), < 50 mg/dL (F); or pharmacologic treatment	≥ 130 mmHg systolic or ≥ 85 mmHg diastolic, or pharmacologic treatment	Fasting glucose ≥ 100 mg/dL or diagnosed T2D
Cook Criteria	--	≥ 90 th percentile	≥ 110 mg/dL	< 40 mg/dL (M&F)	≥ 90 th percentile for age and sex	Fasting glucose ≥ 110 mg/dL

* Adult MetS criteria are used for diagnosis in adolescents age 16 yrs and older

WC = waist circumference; HDL-C = high density lipoprotein cholesterol; T2D = type 2 diabetes; OGTT = oral glucose tolerance test

The use of a cMetS score improves upon the use of specific diagnostic criteria in a number of ways. As previously discussed, the prevalence of MetS identified through the use of the traditional dichotomous criteria is very low (~4%-7%) (20–22). This low prevalence results in decreased statistical power in association studies. Additionally, the use of the dichotomous criteria excludes youth who are at high risk of developing MetS and who may benefit from early intervention to prevent progression of the syndrome. Although the use of a continuous score addresses some limitations of the dichotomous MetS classification, several scores using differing criteria have been reported in the literature (23).

The traditional method of computing cMetS scores is severely limited by the use of sample-specific statistics. These sample-specific cMetS scores have poor external validity because they are specific to the study sample on which they are based, and thus it is difficult to extrapolate findings from individual studies to the population at large. For this same reason, the use of a sample-specific score for specific study groups (i.e. obese adolescents) has limited utility. Using NHANES III data, Cook and colleagues developed growth curves for each feature of MetS and constructed age- and sex-specific percentiles for each variable. These data were also used to identify specific percentile cut points that correspond to the adult NCEP-ATPIII MetS criteria for each variable, which can be used to diagnose MetS in youth (24). The normative data described in the study by Cook can thus be used to create a cMetS score that is based on nationally representative data.

In summary, several definitions of MetS exist for youth, making it difficult to compare the prevalence of MetS between studies. Recent growth curves published by Cook and colleagues (24) allow children and adolescents from the US to be classified based on age- and sex-specific nationally representative data and should increase the accuracy of classification of

MetS in this population. Additionally, these growth curves allow cMetS to be calculated based on national data rather than population-specific data, and allows metabolic risk to be treated as a continuous variable rather than as a dichotomous variable.

Physical Activity and Metabolic Syndrome/Cardiovascular disease in Children

Physical activity has been associated with a number of health benefits and inadequate activity is generally regarded as one of the cornerstone risk factors for overweight and obesity (25). Thus, it is reasonable to believe that PA may be beneficial in the prevention of treatment of overweight, obesity, and related conditions in childhood as well as adulthood.

In general, the results of large cross-sectional studies have demonstrated inverse relationships between habitual physical activity and MetS in children and adolescents. Study methods have varied in terms of measuring PA and classification of MetS and/or CVD risk status but over all, PA appears to be beneficial in reducing cardiovascular risk among youth. Results from the European Youth Heart Study (EYHS), in which physical activity was measured by accelerometry, indicate that time spend in PA is inversely associated with cMetS independently of the effects of cardiorespiratory fitness on the score (26). These findings are supported by those of other large population studies also implementing accelerometry, such as NHANES in the US (27), AFINOS in Spain (28), and another study in Vietnam (29). When PA is measured by self-report, less active youth are still significantly more likely to meet the criteria for MetS than their more active counterparts (30).

Differing intensities of activity may have varying influences on MetS. In the AFINOS study, only vigorous PA was associated with MetS (28). In the EYHS, on the other hand, low-, moderate-, and vigorous-PA were each independently associated with cMetS (26). The differing

results on intensity of activity may be explained, in part, by the relationship between physical activity and cardiorespiratory fitness (CRF). While these terms are not the same they are often used interchangeably, and often the assumption is that increased PA means increased CRF. Studies examining the independent relationships of PA and CRF with MetS have found differing results. In general, those studies that have found only CRF to be independently associated with MetS have used self-reported measures of PA (3). When using objective measures of PA, Ekelund and colleagues found that increasing PA was associated with MetS even beyond the influence of CRF (26). Still others have found that the effect of PA is weakened once adjusted for CRF (28; 31). However, consideration of body fatness may further weaken these associations. In a large study of children and adolescents, Ondrak and colleagues found that CVD risk was associated with aerobic fitness but that percent body fat was a much stronger predictor (32). These results suggest a need to further examine the influence of CRF on MetS and levels of individual variables, independently of body composition. Interestingly, the relationships reported by Ondrak and colleagues were strongest in the youngest children (ages 8-10yrs) and weakest in the oldest (ages 14-16 years) while CVD risk score was highest in those ages 11-13 yrs. This finding supports the notion that adolescents are especially vulnerable to increased levels of CVD risk factors during puberty, and the authors suggested that the dramatic changes in the hormonal environment that occur during puberty might explain the reduced influence of fatness on CVD risk score after this point (32).

Adolescents at highest risk for CVD may benefit more from increased PA and/or CRF than their lower-risk peers (33–35). When comparing quintiles of CRF, Lobelo and colleagues found decreasing CVD risk score with increasing CRF. In fact, the largest decrease in CVD risk was found between the first and second (very low to low) CRF quintiles (35). The results of the

AFINOS study suggest that the relationship between CRF categories may be dependent upon the intensity of habitual PA. Adolescents with lower fitness levels were apparently protected from MetS by participating in more VPA. Therefore, while it is still unclear whether PA or CRF has a greater influence on MetS, and whether the effects of these traits are independent of each other, increasing VPA may be effective for reducing MetS risk among adolescents with low fitness levels (28).

Influence of Diet

As shown in Table 2.3, adolescents typically consume amounts of carbohydrates (including added sugar), total fat, and protein within the Acceptable Macronutrient Distribution Range (AMDR) set by the IOM. However, most fail to meet the recommendations for saturated fat, mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), and fiber (36–38). In terms of micronutrients (Table 2.4), most adolescents do not consume sufficient vitamin A, folate, potassium, calcium, and magnesium, while also consuming much more sodium than recommended (39–42). Overweight and obese adolescents tend to exhibit different dietary patterns than those of their normal weight peers. Specifically, low dairy intake is associated with increased BMI, sum of skinfolds (suprailiac and subscapular) (43), and increased waist circumference (44) in adolescent girls and boys. Low intakes of fruits and vegetables are also inversely related to adiposity, while children who eat more servings of meat tend to have larger waist circumferences (44). In light of these relationships, it is not surprising that overweight and obese adolescents tend to have more adverse CVD risk factor profiles than their normal weight peers.

Table 2.3. Macronutrient intakes of adolescents compared to dietary reference intakes (DRIs)

		Recommended (AMDR)	Actual Intake	Difference (AMDR-Actual)
Carbohydrates	Total (% of kcals)	45-65	52.6 ± 0.4	Within range
	Added Sugars (% of kcals)	< 25%	19.1 ± 0.4	Within range
	Dietary Fiber (g/1000 kcal)	14	6.0 ± 0.1	-60.3%*
Fat	Total (% of kcals)	25-35	33.4 ± 0.34	Within range
	Saturated (% of kcals)	< 10	11.8 ± 0.16	+18%
	Monounsaturated (% of kcals)	Up to 20%	12.6 ± 0.15	-37%
	Polyunsaturated (% of kcals)	10%	6.7 ± 0.21	-33%
Protein		10-30	13.7 ± 0.2	Within range

AMDR: Acceptable Macronutrient Distribution Range. Note: AMDR values are taken from the DRI tables at <http://fnic.nal.usda.gov>, adapted from the DRI reports at www.nap.edu; Recommendation for dietary fiber taken from 2010 Dietary Guidelines for Americans. Reported intakes adapted from Deshmukh-Taskar et al.(37), and Troiano et al. (36).

* Because of differing recommendations, mean reported for both sexes combined was compared to the DRI for each sex separately.

Table 2.4. Intakes of selected vitamins and nutrients related to cardiovascular health.

	Recommended (DRI)			Actual Intake			Difference (DRI-Actual)		
	Both Sexes	Males	Females	Both Sexes	Males	Females	Both Sexes	Males	Females
Vitamins									
Vitamin A (µg/day)	--	900	700	--	874	623	--	-2.9%	-11.0%
Vitamin C (mg/day)	--	75	65	--	94.2	67.6	--	+25.6%	+4.0%
Vitamin E (mg/day)	5	--	--	7.7	--	--	+54%	--	--
Vitamin D (µg/day)	5	--	--	6.7	--	--	+34%	--	--
Folate (µg/day)	400	--	--	231	--	--	-42.2%	--	--
Minerals									
Sodium (mg/day)	1500	--	--	3363	--	--	+124.2%	--	--
Potassium (mg/day)	4700	--	--	2459 ± 43.6	--	--	-47.7%	--	--
Calcium (mg/day)	1300	--	--	1134	--	--	-12.8%	--	--
Magnesium (mg/day)	--	410	360	250 ± 3.9	--	--	--	-39.0% ⁺	-30.6% ⁺
Selenium (µg/day)	55	--	--	81	--	--	+47.3%	--	--

Note: Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) values are taken from the DRI tables at <http://fnic.nal.usda.gov>, adapted from the DRI reports at www.nap.edu. Reported intakes adapted from Lytle et al. (39); Stang et al. (40), Sugiyama et al. (41); and Volpe et al. (42).

Intervention studies examining the influence of dietary patterns on MetS and CVD risk factors in adolescents are limited; therefore much of the information presented here comes from studies involving adults. One of the most notable dietary interventions for reducing CVD risk is the DASH (Dietary Approaches to Stop Hypertension) eating plan. This was the first trial to test the effects of entire patterns of eating, rather than simply reducing sodium intake or supplementing with other nutrients (i.e. magnesium), on lowering blood pressure in hypertensive adults. Participants randomized to the DASH group received dietary and behavior change counseling, and were directed to eat a diet high in fruits and vegetables (approximately 9 servings per day), that included consumption of low-fat dairy products (2 servings per day), and low in sodium (<3000mg/day) and saturated fat (<6% of total calories per day). Subjects followed the prescribed diet for 8 weeks. By the end of the intervention period, the reduction in systolic blood pressure in the DASH group exceeded that of the control group by 11.4mmHg and importantly, also exceeded the reduction in the high fruit and vegetable group by 4.1mmHg. The DASH group also experienced a greater reduction in diastolic blood pressure, though this difference was not as large (-5.5 mmHg in DASH compared to control) (45). These results indicate that following the DASH eating pattern allows for significant reductions in both systolic and diastolic blood pressure even without weight loss.

The DASH diet has also been used to treat other components of MetS among overweight and obese individuals in Tehran, Iran (46). Participants following a hypocaloric DASH diet were compared to a weight reduction group and a control group. After the 6-month long intervention, participants in the DASH group experienced improvements in blood pressure (systolic and diastolic), lipids, triglycerides, and weight, and improvements in this group exceeded those in the weight control group for each component (46). The DASH diet remained superior to the weight

control diet even after controlling statistically for weight loss, a finding that is supported by the previous DASH study, in which blood pressure was improved without a significant change in body weight (45).

Diets that are particularly high in fruit and vegetables (and thus, fiber and select micronutrients) have also been successful in management of type 2 diabetes (T2D) (47) and other CVD risk factors (48). A recent review (47) of the role of vegetarian diets in the treatment of T2D summarizes several observational and intervention studies. Seventh-day Adventists, who tend to lead generally low-risk lifestyles, the prevalence of T2D is about twice as high among non-vegetarians as it is among vegetarians. Intervention studies in which subjects follow low-fat vegetarian (or near-vegetarian) diets have shown improvements in insulin sensitivity, fasting blood glucose, and hemoglobin A1c, to the point that some subjects taking insulin have been able to discontinue its use. Because some of these studies have also included intense exercise programs and have seen rather significant weight loss (7.2 kg in one study), some of the improvement in glycemic control is probably a result of this weight loss. In addition to the improvements in measures related to diabetes, some studies have also found that the adoption of vegetarian diets results in lower LDL cholesterol and higher HDL cholesterol, reducing the risk of cardiovascular events among individuals with adverse lipid profiles (47).

The CARDIA study (Coronary Artery Risk Development in Young Adults) study yielded two papers examining the relationship between dietary factors and MetS or CVD risk factors (48; 49). Subjects in this study were 18-30 years old, and had been followed for 10 years at the time of publication. Dietary habits were assessed using a food frequency questionnaire at study enrollment and also during year seven. In this study, participants who ate more fiber tended to weigh less and to gain less weight over the follow-up period. Fiber intake was also inversely

associated with fasting insulin in both white and black participants. Similar patterns were found for blood pressure and triglycerides, though a large portion of this relationship was explained by the improvements in insulin levels (48). In an additional paper from the same study, dairy intake was also found to be associated with a more favorable insulin resistance syndrome (IRS) profile, but only among overweight subjects (49). As might be expected from the results of the previous paper (48), increased fiber intake was also associated with a more favorable IRS profile. The authors also adjusted for weight gained over the 10-year follow-up period, and found that dairy intake was still associated with lower odds of IRS, although the confidence intervals were somewhat wider in the adjusted model than in the unadjusted model. Interestingly, both low- and high-fat dairy products were associated with reduced odds of IRS. The high content of calcium, magnesium, and potassium in dairy products may be partially responsible for this relationship (49), and it is important to note that those three nutrients are also emphasized in the DASH eating plan (45).

Somewhat in contrast with the findings in adults, Latino adolescents who increased their fiber intake across a 16-week intervention experienced significant reductions in both BMI and visceral fat (measured by MRI), but these reductions did not lead to improvements in blood glucose or insulin levels (50). Those adolescents who reduced their sugar intake did see improvements in insulin secretion, although no change in BMI or visceral fat occurred in this group. These results seem to suggest that changes in visceral fat mass may not have the same positive effect on insulin resistance that is seen in obese adults. However, it is possible that these adolescents experienced other positive changes as a result of this decreased fatness, perhaps in the form of reduced blood pressure or circulating triglycerides, which were not reported in this

paper. More studies are necessary to determine the best dietary intervention to treat MetS during adolescence.

Assessment of Dietary Patterns

Several different dietary assessment methods are available, and each method has a number of strengths and weaknesses. When selecting a dietary assessment tool, it is necessary to weigh the advantages and disadvantages associated with each method in terms of the accuracy of the data obtained, the investment of time, money, and personnel, and subject/respondent burden. Advantages and disadvantages of several common methods are summarized in Table 2.5.

Arguably, the most accurate method of dietary assessment is observation. In this method, a very well trained, unobtrusive observer watches individuals (usually at meal-time) and is able to quantify food intakes. This is often done in school lunchrooms or similarly controlled settings, and thus it is possible to obtain detailed information about the foods consumed, including preparation, condiments, and other details. Although the observation method is accurate, it is very time-consuming and expensive, since trained observers are required. Also, the observer has to take great care to be unobtrusive, so that the subjects do not alter their eating behaviors (51). Another very accurate but expensive and time-consuming option is diet history. In this case, a trained interviewer talks with the subject about his or her usual dietary intake (recent and long-term). The interview format allows for the collection of very detailed data and any unclear answers can be clarified right away, reducing confusion or misinterpretation on the part of the interviewer. Food frequency questionnaires (FFQ) can also assess long- or short-term dietary intakes, though these can be self-administered and require significantly less investment of staff time. The time period for FFQs can vary from a week-by-week basis up to a year.

Table 2.5. Advantages and disadvantages of several common dietary assessment methods (51)(135).

Method	Advantages	Disadvantages
24-hour recall	<ul style="list-style-type: none"> - Low subject burden - Can be performed quickly - Accurate for estimating intakes at the group level with just one recall - Doesn't cause subjects to change their behaviors 	<ul style="list-style-type: none"> - Requires trained interviewer - Time intensive for interviewer, can be expensive - Not appropriate for estimating individual intakes (with just one day) - Requires good memory - Portion sizes may not be estimated accurately
Food record	<ul style="list-style-type: none"> - Specific time-period - Doesn't rely on memory (if foods are recorded right away) - Subjects can be trained in groups, reducing time investment of research staff - One day provides accurate information for group-wide estimates of dietary intake 	<ul style="list-style-type: none"> - Higher subject burden - Subjects may alter dietary patterns - Recording for several days may result in reduced validity - Large volume of data, expensive to code
Food Frequency Questionnaire	<ul style="list-style-type: none"> - Doesn't require trained interviewers - Can be self-administered or interviewer-assisted - Not affected by alterations in habits - Can assess the diet as a whole, or select nutrients or foods - Inexpensive - Low subject burden 	<ul style="list-style-type: none"> - Heavily reliant on subject memory of dietary patterns - Recall period is imprecise - Quantification is imprecise – portion sizes, frequency of consumption - Food descriptions aren't as detailed as with diet records or recalls
Diet History Questionnaire	<ul style="list-style-type: none"> - Not affected by altered habits - Can get very detailed information about foods, preparation methods, etc. 	<ul style="list-style-type: none"> - Requires highly trained interviewers - Very high subject burden, interview can take a couple hours - Recall period and portions may be imprecise - Requires a lot of staff time, can be expensive
Observation	<ul style="list-style-type: none"> - Not affected by altered habits - Doesn't rely on memory at all - Very low subject burden - Defined time period - Possible to measure portion sizes 	<ul style="list-style-type: none"> - Requires highly trained observers - Expensive – requires a lot of staff time

Two other common dietary assessment methods are diet records and dietary recalls (52; 53). Diet records can include data from as little as one day, or can be kept continuously for indefinite periods of time. Frequently, several single-day records are used to estimate overall patterns, though several days (sometimes a month or more) are necessary to accurately quantify intakes of some micronutrients (54). Recalls are generally performed over the phone, and typically cover a single day of food intake (24 hours). With both diet records and recalls it is possible to gather detailed information about foods and drinks, including preparation methods.

With these advantages and limitations in mind, the 3-day diet record method is a good choice to reasonably estimate dietary habits among 12-18 year-old adolescents. Collecting three days worth of records should increase the validity of the data obtained while keeping the subject and data-entry burden relatively low. While recording intake for a week or more might provide a more accurate assessment of intake of some nutrients (especially micronutrients) (54), it is important to balance that with the increased subject burden and the chance that subjects might not record data as accurately when required to do so over an extended period of time (51). Another option would be to perform several 24-hour recalls, though researcher burden would be increased in this case.

Several steps can be taken to improve the quality of the data obtained using this method. First, it is important to make sure the subject is well trained in the method and understands the importance of recording absolutely everything consumed, along with preparation methods, condiments, and anything else added to the food. Providing a pocket-sized record the subject could carry on their person throughout the day may increase compliance. Collecting data on two weekdays on which the subject follows different schedules (i.e. Monday and Thursday) and one weekend day should allow for differences that occur on a daily basis, such as evening activities

or sleeping in on the weekends. To aid in the reporting of portion sizes, the subjects can be given prompts (such as pictures or descriptions) to help them remember what constitutes a serving of different foods and estimate the amount of each item eaten. Researchers should contact subjects to clarify any unclear entries in order to minimize error in data entry and quantification of nutrient intakes. Additionally, the data should be analyzed using well-known nutrition software that is generally accepted as accurate.

The Healthy Eating Index (HEI) was originally developed in 1995 by Kennedy and colleagues (55), in an attempt to design a single index of dietary quality based on US dietary guidelines. The HEI can be used either to assess dietary quality or to measure changes in dietary quality over time. A total of 10 components are included in the scale; each of these 10 components is scored on a range from 0-10, so that subjects receive a diet quality score ranging from 0-100, with a higher score indicating superior dietary quality. The ten components making up the HEI include the five major food groups (fruits, vegetables, grains, dairy, and meat), total dietary fat (percent of total kilocalories), saturated fat intake (percent of total kilocalories), cholesterol intake, sodium intake, and dietary variety. Scores for the first five components, the major food groups, are based on the age- and sex-specific total daily serving recommendations for each individual. The two components dealing with fat intake, components 6 and 7, are scored based on the daily recommendations for each nutrient, such that individuals consuming less than the recommended daily intake receive a score of 10. Components 8 and 9, cholesterol and sodium, are based on milligrams consumed per day. Finally, dietary variety is assessed by counting foods consumed at a minimum of one-half serving in each food group and equivalents are added to determine how many different foods each individual consumed. A perfect score of 10 is assigned to this category if an individual consumes at least 16 different foods over the 3-

day study period (55). In 2008, the HEI criteria were updated to reflect the 2005 USDA dietary guidelines (56). These new guidelines, HEI-2005, reflect both total and whole fruit intake, total vegetables and individual types of vegetables (i.e. dark green leafy vegetables), total and whole grains, milk, meat and beans, oils, saturated fat, sodium, and calories from solid fats, alcohol, and added sugars.

Stress

The term “stress” is generally used in a broad sense to describe any physical, environmental, psychological, or physiological challenge requiring adaptation (57). The human stress response was first characterized by Walter Cannon, who coined the term “fight-or-flight” to describe the endocrine changes that occur to prepare the body to deal with acute danger (58). Later, Hans Selye suggested that the body responds and adapts to stressors through a process consisting of three phases, which he termed the General Adaptation Syndrome (GAS) (59). More recently, the terms “allostasis” and “allostatic load” have been used to describe the body’s adjustments and adaptations to challenges (57).

Selye suggested that the body responds to stressors in a non-specific way (59). Thus, the assumption was that the body responds in similar ways to physical threats, such as a serious injury, and perceived threats, such as a final exam or the loss of a job. More recently, however, the importance of the individual’s subjective appraisal of stress has received more attention. As originally suggested by Lazarus (60), the stress response can vary greatly based on the way in which a stressor is perceived. In adults, neighborhood problems such as crime, violence, and unemployment have been associated with increased psychological distress and poor health behaviors, even after adjustment for neighborhood SES, age, sex, and other confounders (61). These results highlight the importance of perception of stressors, suggesting that people from

lower-income areas and neighborhoods with more crime and violence interpret potential stressors in a more threatening way.

Several instruments have been used to assess perceived stress in a number of populations. Many of these instruments were developed for use in adults and adapted for use with children. Additionally, studies implementing paper-and-pencil instruments for use with children have been conducted in fairly homogenous groups and have not considered the unique experiences of an ethnically and economically diverse group of individuals (62).

Assessment of Psychosocial Stress

Stress Inventories. Stress inventories or checklists can be generalized, asking about a wide range of potential stressors, or more specialized and focusing on specific issues. Inventories are often limited by individual interpretations of threats, inclusion of limited stressor items, and a lack of detailed information about an individual's perception of each stressor.

Perceived Stress Scale (PSS). The PSS was originally developed for use with adults by Cohen and colleagues in 1983 (63). This inventory was designed to provide an appraisal of stress and coping over the preceding month. A full, 10-item scale and a shorter 4-item version are both available, however the longer version is more reliable and valid. Each item is rated on a 5-point Likert scale (never, rarely, sometimes, often, and very often). One example question is "How often have you felt that you could not control the important things in your life?" The score from all items are scored to create a total perceived stress score ranging from 0-40 for the long version or 0-16 for the short version (63).

Adolescent Stress Questionnaire (ASQ). Originally developed in 1993, the ASQ was developed in focus groups of adolescents in school years 7-12 (64). The questionnaire was

updated in 2006 to include stressors that are more appropriate for today's adolescents. Ten scales with a total of 58 items are included in the questionnaire.

Adolescent Perceived Microsystems Scales (APMS). Many inventories used in youth studies have either been originally designed for use with adults, or have been developed using focus groups from specific ethnic and SES groups, often limiting their generalizability to lower SES groups and minorities. The APMS (62) was developed specifically for a culturally diverse, urban population, using focus groups from three urban areas (Baltimore, MD; Washington, DC; and New York, NY). The original sample was 26% black, 26% white, 37% Latino, and 59% female, extending the applicability of the questionnaire to these groups. The APMS provides an assessment of an adolescent's perception of his or her family, peer, school, and neighborhood microsystems. These microsystems are assessed on three different scales:

- Social support: An adolescent's family, peer, and school relationships.
- Daily hassles: school performance, family responsibilities, problems in the neighborhood
- Involvement: extracurricular school activities, leisure, recreational activities, neighborhood involvement

Pediatric Symptoms Checklist. The Pediatric Symptoms Checklist (PSC) is a 35-item questionnaire designed for use by pediatric practitioners to detect psychosocial problems among their patients (65). For each item, the child (or a parent) indicates whether the statement describes the child never, sometimes, or often. Each item is scored based on the response and these scores are added to compute a total score. Used primarily with chronically ill children, the PSC has been implemented to identify symptoms of internalizing and externalizing difficulties and other psychological distress in a primary care (66) and/or chronically ill patients (67; 68).

Validity and reliability of the PSC has been demonstrated in children with varying degrees and types of psychological problems (69). Based on this information, the PSC is the instrument used by clinical staff at the Helen DeVos Children's Hospital Healthy Weight Center (HWC), and is the instrument that will be used to assess psychosocial stress in this study. Staff at the HWC have communicated with the developer of the PSC and have identified subscales indicative of depression and anxiety.

Interviews. Interviews improve upon some of the weaknesses of questionnaires in that they are more able to distinguish context and an individual's interpretation of a given stressor.

Life Events and Difficulties Schedule (LEDS). Originally developed for adults, the LEDS is administered by trained interviewers and scored by separate raters who are blind to the individual's subjective responses. Stressfulness of specific events are rated based on the event context and past experiences, long-term threat, timing of the event, and whether the event is focused on the individual or on others. The LEDS also differentiates between acute stressors, termed events, and chronic stressors, termed difficulties (70).

Standardized Event Rating System (SEPRATE). The SEPRATE is more specific than the LEDS, and is made up of yes/no questions regarding 84 standardized stressors. Any "yes" answers are further probed with additional questions in order to identify the magnitude of change induced by the event, desirability of the event, disruptiveness to daily routines, threat to life, and the degree of the individual's control over the event. As in the LEDS, raters are blind to social vulnerability and subjective responses.

In summary, many methods exist to assess stress using either surveys or interviews. Surveys can evaluate a wide range of stressors or be very specific, but can be limited in their ability to

distinguish context and individual reactions to stressors. Interviews improve upon these weaknesses in that they often are more able to distinguish context and interpretations of stressors, but they may be more susceptible to interviewer bias. This bias can be reduced through interviewer training. The method selected should be appropriate for the subject population (i.e., have been developed for individuals of similar age and social situation) and as free as possible of bias. The PSC (youth report) is the instrument used by clinical staff at the Helen DeVos Children's Hospital Healthy Weight Center (HWC), and is the instrument used to assess psychosocial stress in this study.

Psychosocial Function

The term “psychosocial functioning” has been used in a very broad sense, often encompassing peer and family relationships, health-related quality of life (HRQoL), school performance, and mental health. In most cases, psychosocial functioning describes the way individuals interact with and relate to others and to themselves. It can be defined as “...the ability to cope and tolerate stress, and the capacity for developing a value and belief system.” (71). In this sense, optimal psychosocial functioning is necessary in order to adequately cope with stressors and threats that are encountered on a daily basis. An individual with poor psychosocial functioning might lack supportive peer relationships, have poor self esteem, or experience depression, anxiety, or other mental health problems, especially when faced with stressors such as difficulties in school or disruption of the family home environment. Conversely, an individual with good psychosocial functioning would be able to successfully cope with these stressors without experiencing a major disruption in day-to-day functioning.

Psychosocial factors that have been associated with the onset of pediatric obesity include interpersonal problems with peers, divorce in the immediate family, personal medical concerns,

and a variety of other peer and family factors. Many of these are also frequently named as barriers to healthy behaviors among obese youth, especially in terms of the ability to make healthy lifestyle changes (72). Studies of psychosocial function among obese youth generally consider experiences with bullying, adversity such as parental divorce and family uncertainties, and periods of major depressive disorder and other acute psychiatric difficulties (73–75). Many of these studies in both children and adults focus on HRQoL as a marker of psychosocial function (76; 77).

Physical activity and participation in leisure time activity are associated with improved HRQoL among both children and adults, and these findings are consistent within both healthy and chronically ill populations. Among adults with prediabetes, individuals who meet PA recommendations score better on HRQoL assessments than those who do not meet recommendations. More specifically, improved HRQoL is associated with increased MET*min/week of PA, maintenance of a healthy BMI, and avoidance of smoking (76). Other factors that have been associated with improved HRQoL among adults include lower blood pressure, waist circumference, perception of better physical function, and total cortisol. Furthermore, participation in LTPA is associated with improvement in both psychosocial and physical measures of well-being, including higher levels of positive psychosocial states (i.e. vigor, well-being, and calm) and reduced frequency of depression and negative affect (78).

In obese adolescents, a higher BMI and increasing number of comorbidities is associated with decreasing HRQoL (77). Participants in this study were seeking treatment for pediatric MetS clinic, and thus experienced at least two of the components of MetS. Health related quality of life was assessed using the Impact of Weight on Quality of Life-Kids (IWQOL-Kids) questionnaire, and assessed the domains of body esteem, social life, family relationships,

physical comfort, and a total score. Scores on both the youth and parent report forms of the IWQOL-Kids were inversely associated with BMI and BMI z-score (all subscales), indicating that HRQoL decreased with increasing levels of obesity. Additionally, the number of comorbidities a participant had varied with the total score and subscale scores on the IWQOL-Kids, such that participants with more comorbidities had higher scores on the inventory. Female participants and non-Hispanic white participants experienced poorer quality of life, even after consideration of BMI, indicating that females and non-Hispanics may experience more of the negative psychosocial affects of obesity (77).

Biomarkers of the Stress Response

The stress system, made up of the sympathetic nervous system (SNS) and the HPA axis. Stressors may be of either internal or external origin, and may be physical, psychological, chemical, social or biological. Exposure to a stressor stimulates both the SNS and HPA axis to regulate changes in metabolism, circulation, and respiration in order to prepare the body for intense physical activity in emergency situations (79).

Activation of the SNS is termed the “fight or flight” response, initially described by Walter Cannon (58). Exposure to a stressor and activation of the SNS causes increased secretion of epinephrine and norepinephrine into the peripheral circulation, and results in increases in heart rate (HR), blood pressure (BP), blood glucose levels, blood flow to the skeletal muscles, and bronchodilation. SNS activation also results in suppression of growth, reproduction, and digestion.

While SNS activation is the body’s initial response to an acute stressor, prolonged stressors result in activation of the HPA axis. When stimulated, corticotropin-releasing

hormone (CRH) is released from the hypothalamus and stimulates the pituitary gland to release adrenocorticotrophic hormone (ACTH) into the blood stream. Circulating ACTH stimulates release of cortisol from the adrenal cortex. Increases in circulating cortisol then cause metabolic changes throughout the body, promoting glycogenolysis and lipolysis in the skeletal muscle and liver, as well as protein catabolism and gluconeogenesis from non-carbohydrate precursors.

Integration of SNS and HPA axis activation allows the body to properly respond to both acute and chronic stressors. However, chronic over-activation of these systems can have detrimental effects. The work of McEwen and Bjorntorp highlights the detrimental effects of chronic HPA and SNS activation, which include increased susceptibility to dementia and depression (79), hypertension (80), and abdominal obesity (80). Because activation of the SNS and HPA axis results in changes in circulating hormones, these hormones are often used as biomarkers of the stress response. Cortisol is one of the most common biomarkers used for this purpose.

When the HPA axis is functioning normally, cortisol is released into the circulation in a predictable diurnal pattern. Cortisol release peaks early in the morning, approximately 30 minutes after waking and falls throughout the day. Normal HPA axis activity can be characterized by pulsatile release of both ACTH and cortisol over the course of the day. The cortisol peak seen early in the day is due to more frequent, stronger pulses of ACTH and cortisol release. Activity of the HPA axis is regulated primarily through a negative feedback system, so that high levels of circulating cortisol inhibit the release of ACTH from the pituitary, and high levels of ACTH suppress CRH release from the hypothalamus. This negative feedback control limits tissue exposure to cortisol, thus regulating cortisol's effects on metabolism and other physiological functions. Other factors that may alter HPA axis activity include other hormones

and cytokines (81). Acute stressors, such as a meal, physical activity, or mental stressors stimulate the release of cortisol, such that the normal diurnal pattern can be altered at any time throughout the day.

Once released into the blood, cortisol circulates bound to corticosteroid-binding globulin (CBG) and a small amount circulates freely in solution (about 10%) or bound to albumin (about 15%). Plasma levels of CBG are increased in high estrogen states (i.e. pregnancy) or in the presence of other endocrine conditions, such as hyperthyroidism or diabetes. Conversely, plasma CBG is reduced in cases of hypothyroidism; genetics also play a role in either increased or decreased plasma CBG concentration. At the target tissues, cortisol binds to glucocorticoid receptors within cells, and this cortisol-receptor complex is responsible for most of the actions of cortisol in the body. In fact, differences in glucocorticoid receptor gene expression may explain some race and gender differences in cortisol levels (82).

Once it reaches the tissue, the action of cortisol is controlled by several different enzymes. One of these enzymes, 11 β -hydroxysteroid dehydrogenase (11 β -HSD) exists in two isoforms. The first isoform, 11 β -HSD type 1 (11 β -HSD1) is present in both the liver and adipose tissue and is responsible for reactivating cortisone to its active form, cortisol. The second isoform, 11 β -HSD type 2 (11 β -HSD2), is found in the kidney and converts cortisol to its inactive form, cortisone. When functioning properly, the action of 11 β -HSD types 1 and 2 help to control the effects of cortisol at the tissue level, ensuring adequate levels within tissues depending on cortisol for metabolic control (liver and adipose tissue) and inactivating excess cortisol in the circulation (83). Tissue-specific alterations in the activity of 11 β -HSD1 have been documented in obese men (83) and obese pubertal adolescents (84). In these groups, reactivation of cortisone to cortisol is increased within adipose tissue while it is simultaneously reduced

within the liver, resulting in increased excretion of cortisol metabolites in urine in concert with increased concentrations of cortisol within adipose tissue. As suggested by Wiegand and colleagues (84), this altered enzyme activity may protect individuals from increased circulating cortisol levels and the concomitant hyperinsulinemia and insulin resistance that has been demonstrated in individuals with Cushing's syndrome.

Traditionally, cortisol responses to acute stressors and diurnal variation in cortisol levels have been measured in serum either via a single venipuncture or repeated samples drawn from a venous catheter. Because cortisol is released in response to stress, serum measures may be subject to error. More recently, saliva sampling has been used to assess these parameters. Cortisol levels in saliva have been found to accurately reflect serum free-cortisol levels (that not bound to cortisol-binding globulin) (85), which is often thought to be the more biologically active portion. Saliva collection is non-invasive and allows researchers to avoid the additional stress of venipuncture. Additionally, saliva collection does not need to be done in a laboratory, allowing for analysis of cortisol levels in normal daily living situations. Analysis of saliva samples can be done via enzyme-linked immuno-absorbent assay (ELISA) techniques, avoiding the potential radiation exposure necessary to analyzed serum cortisol values via radioimmunoassay (RIA).

Though the availability of technologies to analyze cortisol levels in saliva have made assessment of cortisol in free-living situations and among all age groups significantly more feasible, several factors must be considered when designing studies.

Endocrine Changes During Puberty. The onset of puberty and maturation of the hypothalamic-pituitary-gonadal (HPG) axis, resulting in the attainment of sexual maturation, are

the major characteristic of the adolescent period. At the onset of puberty, pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates release of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland (86). The frequency of release of the gonadotropins is modified by circulating steroid hormones – in fact, circulating cortisol is known to decrease the LH response to GnRH in both males and females (82). Ultimately, the gonads (testes in boys, ovaries in girls) begin to produce larger quantities of sex steroids (testosterone in boys, estradiol and progesterone in girls) (86). These changes in sex hormone release, as well as increased release of dehydroepiandrosterone-sulfate (DHEA-S) and growth hormone result in the rapid gain in height and changes in body composition that are characteristic of the adolescent growth spurt (87).

Maturity-Related Variation in HPA Axis Activity

Infancy. Before the advent of technology to measure cortisol levels in saliva, assessment of HPA axis in infants was conducted largely in animals or relied on use analysis of urinary cortisol and its metabolites. The adoption of salivary cortisol assessment has greatly increased the number of studies of both basal and stress-related activity of the infant HPA axis (88).

Newborn infants exhibit low levels of total cortisol in serum, and the concentration of cortisol increases over the first year parallel to increases in the concentration of CBG. Cortisol in saliva, however, shows a different pattern, with similar levels in the newborn compared to later in the first year (88) – this difference is because salivary levels of cortisol reflect the amount of free cortisol in serum, which is largely unaffected by CBG levels as discussed previously. The early morning peak in cortisol levels is evident as early as seven weeks of age in about 30% of infants, and in almost all infants by 12 weeks (89). Additionally, cortisol levels increase after feeding (post-prandial increase) in infants as young as 9 months (88; 90). Because of this, it is

important to control for the time of cortisol sampling beginning with very young infants, especially if sampling is to occur in the morning when cortisol levels are changing quickly (88). Other factors that should be controlled in studies include maternal use of medications and/or caffeine (for breast-fed infants), the timing of naps and car rides (90), and gestational age (88). Between the ages of 12- and 18-months, correlations have been found between waking time and baseline cortisol levels, as well as with percent change (with respect to baseline) in cortisol levels in response to stress though baseline levels themselves may not be correlated with the stress response (90), a finding that differs from those in older children (91).

Childhood and Adolescence. Ease of sampling and the non-invasive nature of saliva collection have made this method very popular for cortisol research in young children. However, some characteristics of cortisol secretion, and of children's behavior, need to be considered when designing studies in this age group (92).

In order to collect useful cortisol data in all age groups, it is necessary to standardize the collection time, use consistent procedures and materials, and control for food, drinks, medications, and other external factors that may affect cortisol levels. The use of a salivette, or cotton swab-like collection device, may not be appropriate for young children. Because of this, other methods have been developed for use in this age group. The "passive drool" technique involves asking a child to spit into a collection tube through a short straw. Plastic syringes have also been used to suction saliva from the child's mouth, and other investigators have used cotton swabs that an adult can hold firmly in order to avoid any potential choking hazard. Occasionally, researchers have used powdered drink mix, a small amount of sweetener, or sugarless gum to stimulate saliva flow. Because cortisol assays may be affected by the pH of saliva, it is important to use stimulants that have been identified by the specific assay's manufacturer (92).

Many of the studies examining HPA axis changes during childhood and adolescence are designed to examine the relationships between either basal or stress-related cortisol secretion and mental health concerns, such as depression and anxiety (91; 93–95) . These studies vary in cortisol sampling methods (i.e. time of day), assessment of chronic and acute stressors, and evaluation of pubertal status. As a result, it is difficult to draw definitive conclusions regarding any changes in HPA axis activity in these age groups.

Studies examining normal cortisol levels and responses to stress in children have been largely cross-sectional in nature and vary in methodology and the ages of children included in each study. The majority of studies aim to determine whether a gender difference exists prior to the onset of puberty.

Sex-Related Differences in HPA Axis Activity

In a study of 5 year-old children (91), cortisol was assessed upon wakening and 10-, 20-, and 30-minutes post-awakening indicated that girls exhibited higher levels of cortisol at all time points. In addition, girls were more likely to be high-secretors of cortisol and had higher stress-induced levels of cortisol, suggesting that children with higher baseline HPA activity are also more responsive to stress. Interestingly, no awakening response was found in the boys participating in this study, which is in contrast to other studies involving children of varying ages (96). These results are supported by a study of older children (10-12 yrs), in which girls exhibited higher cortisol levels than boys upon wakening and 30-minutes later, beginning at pubertal stage 1 (96). An additional study by Tornhage and colleagues (97) is more difficult to compare to the others because saliva samples were collected between 8:00 and 9:00 AM while the children were in school, thus missing the waking value and response to waking. Regardless, these researchers also found a sex difference in basal cortisol levels, though in this case 7 year-

old boys exhibited higher cortisol levels than girls of the same age. The authors suggested that the higher levels seen in these boys might be related to adrenarche (97), at which time increased ACTH secretion stimulates release of both cortisol and the adrenal androgens DHEA and androstenedione (86). Two other studies comparing basal cortisol levels between pre-pubertal boys and girls have not found any differences between the two sexes (98; 99), though Knutsson et al. did find a slight sex difference at pubertal stage 2 (girls > boys); however, cortisol in their study was measured in serum, limiting the ability to compare these results to those of other investigators. Due to the methodological differences in timing of sampling and the use of salivary vs. serum cortisol, it is not yet clear whether a gender difference in basal cortisol levels exists prior to the onset of puberty.

It has been suggested that a sexual dimorphism in HPA axis activity may emerge around the time of puberty. Most studies of older children and adolescents do support the notion of gender differences within this age group, though some have not found any differences in basal cortisol levels (95; 99). Studies by Tornhage (97), Netherton (98), Kelly (100), and Bouma (101) have found differences between older boys and girls though again, the ability to compare between studies is limited by methodological differences. In the study by Tornhage et al. (97), in which saliva samples were collected after the start of the school day, 11- and 14-year-old girls exhibited greater cortisol levels than boys of the same age. Samples were also collected an average of 2 hours after waking in the study by Kelly and colleagues (100) and again, girls in this study exhibited higher cortisol levels compared to boys, though in this case the sex difference disappeared when samples were collected 30-minutes later. Samples were also collected at 8:00 AM in Netherton's study (98), though in this case the timing of sampling with respect to awakening is unclear. Again, mid-to post-pubertal girls exhibited greater cortisol

levels than mid- to post-pubertal boys, such that levels in girls were approximately 20-30% greater than those in boys (98). The only study to sample cortisol immediately upon waking in adolescents was that by Bouma and colleagues (101). In this case, girls were again found to have greater cortisol levels than boys, though the awakening response (determined from samples collected 30-minutes later) did not differ by gender. It has been suggested that the sex difference emerging around puberty may be related to interactions between cortisol and sex hormones secreted by the HPG axis (98), and this is supported by the results of animal studies discussed in the following section.

Differences by Stages of Pubertal Development. Several of the studies that compared basal cortisol levels between sexes also compared children and adolescents classified by stages of pubertal development. Unfortunately, not all studies include an assessment of pubertal staging, and those that did include staging did not always clearly define the classification scheme used. The studies by Rosmalen (96), and Netherton (98) used sketches of secondary sex characteristics described by Tanner and Whitehouse (102) for either self- or parent-assessment of development, though neither study specified whether criteria were based on pubic hair, breast, or genital development. Pubertal development was assessed according to Tanner's criteria for pubic hair and breast development and by Prader testicular volume in the study by Knutsson et al. (99), though it is unclear whether this was done by physician- or self-assessment. The studies by Tornhage et al. (97), Gunnar et al. (95), and Matchock et al. (103) do not provide any detail regarding their criteria for pubertal staging.

Similarly to the study by Hatzinger discussed previously (91), Rosmalen's study involved the collection of a saliva sample immediately upon waking and a second sample 30-minutes later; a third sample was collected at 8:00 PM (96). In this sample, approximately 85% of

children were classified as either pubertal stage 1 or 2 and very few children were sexually mature. No differences were found according to pubertal stage, such that no change in HPA axis activity was evident as children became more sexually mature (96). It is important to remember, however, that the cross-sectional study design limits the ability to draw conclusions on the changes in HPA axis activity across pubertal development. The majority of children participating in this study (71%) did exhibit a cortisol awakening response and values tended to be higher in the summer months than the other months of the year, a difference the authors suggested might be related to participation in different activities (96).

In addition to comparing subjects by age, Tornhage and colleagues also classified subjects according to pubertal stage and compared cortisol levels across the stages. The criteria for pubertal staging are not given in the paper; however, a peak in basal cortisol levels was noted for both sexes in stages 2 and 3, with levels in stages 4 and 5 declining to prepubertal levels in boys but remaining elevated in girls (97). Without more information about pubertal staging, however, it is difficult to compare these results to those of other studies.

An additional study by Netherton and colleagues (98) also included children and adolescents across the transition to adolescents (8-16 y, mean age 12.8 y). Participants were classified into groups according to pubertal status similarly to the study by Rosmalen (96). Two groups were then created, grouping children in stage 1 and stage 2 into a pre- to early-pubertal group (PEP) and those in or beyond stage 3 in a mid- to post-pubertal group (MPP); the majority of the sample (approximately 70%) were in the MPP group. Although a significant positive association was found between age and mean cortisol in girls, when PEP and MPP girls were compared no differences were found, and the effect of age disappeared. No association was found between mean cortisol and either age or pubertal status among boys in this study (98).

Studies by Matchock et al. (103) and Gunnar et al. (95) also suggest that the sexual dimorphism evident during adolescence may be dependent upon pubertal status. In the study by Gunnar and colleagues, 13 year-old girls exhibited a greater cortisol response to a laboratory stressor than boys of the same age, however, the girls were more pubertally advanced at that age than the boys (95). Similarly, the study by Matchock and colleagues indicates a later peak in boys (and an earlier peak in girls) in the later stages of puberty (103); this difference in peak time was the only sex difference evident in the Matchock study. Unfortunately, neither of these papers included information regarding classification of pubertal status.

In a study of adolescent girls, Oskis and colleagues (104) used menarcheal status as an indicator of maturity and compared daily cortisol patterns during the different phases of the menstrual cycle. Post-menarcheal girls peaked later in the morning (45-min post awakening vs. 30-min post awakening) and had higher cortisol levels over the course of the day; however, this relationship disappeared after the analyses were controlled for BMI and age. The authors concluded that pubertal status, age, and BMI are all interrelated variables that should be statistically controlled in studies of adolescents, highlighting a weakness in other studies cited here (104). These reported differences in the timing of the morning peak between boys and girls in later puberty (103; 104) may be one reason for the sexual dimorphism that seems to be apparent around puberty. It is possible that cortisol sampling may capture the morning peak in either boys or girls while missing the peak in the other gender; this is further complicated by the lack of coherence in sampling methods between studies.

All of the studies here are limited by the fact that they include cross-sectional data only. This makes it extremely difficult to compare across age or pubertal stage, as it is not possible to know whether any apparent differences come from puberty itself or from individual variation.

One study, performed by Knutsson et al. (99) did include longitudinal data. These data help to clarify the patterns of cortisol secretion across childhood and adolescence; however, cortisol was measured in serum, making it more difficult to compare to other studies. As discussed previously, salivary cortisol reflects only free cortisol concentrations. While this is generally seen as an advantage because it allows for assessment of the most biologically active form of cortisol, serum measurements capture cortisol circulating free in serum and also that bound to CBG and albumin (85). Of a total of 235 children and adolescents participating in this study, 28 were followed longitudinally (2-7 repeated measurements over 0.5-8.0 y). Children spent the night in a hospital and cortisol concentration was measured in serum, first at 1400h and then at 1800h, 2200h, 0200h, 0400h, 0600h, and 1000h, providing a profile of the full daily pattern of circulating cortisol. The longitudinal analysis revealed stable cortisol patterns across childhood and adolescence with wide inter-individual variability. Importantly, no differences were found by pubertal stage in the longitudinal analysis (99).

Animal Studies. Studies of rats indicate a change in the basal activity of the HPA axis and in the stress response around the time of puberty. As in humans, increased levels of GnRH are released by the HPG axis, ultimately increasing production and release of testosterone in males and estradiol and progesterin in females. In males, this increase in testosterone decreases HPA axis stress reactivity while in females, increased release of the female sex hormones increases stress reactivity (105). Similarly, the work of Carey and colleagues (106) indicates that exposure to exogenous estrogens increases basal HPA axis activity of adult female rats. This work was furthered by a 2009 study by Evuarherhe and colleagues (107) in which estrogen levels were altered in both pre-pubertal and ovariectomized rats. Pre-pubertal rats that received an injection of estradiol did not differ from those given an injection of cholesterol in terms of

basal corticosterone concentrations; however, the pre-pubertal rats exposed to estradiol had reduced stress reactivity than did those exposed only to cholesterol. Interestingly, when female rats were ovariectomized prior to puberty and given an injection of estradiol in adulthood, they had a greater stress response and greater basal activity than did rats that had only a sham surgery. These results suggest a shift in the response of the HPA axis to estrogens around puberty that is caused by something other than sex steroids themselves (107). Researchers have suggested that this reversal may be in response to other changes occurring around the time of puberty, such as maturation of the HPA axis negative feedback system through changes in glucocorticoid receptor mRNA expression in the brain (105).

Cortisol and Adiposity

Stress is thought to influence weight gain through several mechanisms. Some of these are behavioral and emotional effects like “comfort eating” and decreased physical activity, while others may be related to hormonal changes in energy homeostasis and appetite regulation. Studies have frequently used socioeconomic status, allostatic load, and cortisol as indicators of chronic stress.

To date, studies in youth examining relationships between stress and cortisol, using socioeconomic status as an indicator of chronic stress, have shown varied results. Some of these studies suggest a positive relationship between stress and cortisol, while others do not. Methodological variation may explain some of these differences. Additionally, age-related differences may make it more difficult to reach a clear conclusion (108).

Recent studies have associated health disparities, including increased rates of childhood overweight and obesity, with socioeconomic status (SES) and indicated that differences in SES

may contribute some of the differences seen among ethnicities (109). Children from low SES families are at a greater risk of becoming overweight (110). Possible contributing factors may include unsafe neighborhoods and resultant physical inactivity, reduced access to safe places to play such as community centers and safe parks, grocery stores (111), and reduced school resources (112). In addition to potentially decreased PA, children from a lower SES background may experience increased stress (113) and may be more vigilant than their higher-SES peers in response to potential threats (114). This suggestion is supported by studies in adults indicating that increased stress is associated with increased adiposity, especially around the abdomen (80; 115).

Physical, psychological, and physiological stressors stimulate cortisol production and release. Cortisol is a glucocorticoid hormone regulated through negative feedback of the HPA axis. In response to an appropriate stressor, such as a drop in blood glucose, CRH is secreted from the hypothalamus, which then causes the release of ACTH from the anterior pituitary. Increased circulating levels of ACTH then stimulate the release of cortisol from the adrenal cortex. Cortisol then travels to the body tissues and mediates several physiological effects, such as the breakdown of triglycerides in adipose tissue to free fatty acids and glycerol. In addition, cortisol activates lipoprotein lipase release from adipose tissue, which promotes storage of lipids in adipocytes (80). Circulating levels of cortisol in the blood follow a circadian pattern, reaching their peak early in the morning, falling in the evening, and rising again towards midnight (116). Food ingestion, exercise, and psychological stressors additionally modify cortisol release.

Disruption of normal HPA axis activity can lead to changes in the diurnal rhythms mentioned above, and increased HPA axis activity can inhibit the gonadal and growth hormone axes, further disposing individuals to visceral fat accumulation (80). This has been supported by

research of Cushing's syndrome, in which it has been shown that the elevated levels of cortisol associated with Cushing's syndrome are associated with increased amounts of visceral adipose tissue (80).

Several studies have examined cortisol levels upon waking, both in blood and saliva, when cortisol levels are typically near their peak. Recently, more attention has been turned to associations between cortisol and adiposity in children and adolescents. Two recent studies have examined the relationships between cortisol levels and adiposity (117; 118). Both studies found significant positive relationships between serum cortisol and abdominal fat. Additionally, Barat et al. found whole body fat mass was not correlated with cortisol levels, however, trunk fat was positively correlated with morning serum cortisol in the total study population (118). Serum cortisol was associated with insulin resistance in both studies. Weigensberg and colleagues also found a positive relationship between morning cortisol levels and the number of features of MetS exhibited (117). Interestingly, when cortisol levels were adjusted for intra-abdominal adipose tissue (IAAT), the relationships were no longer significant, and cortisol was positively related to IAAT, indicating that cortisol is related to abdominal adiposity in overweight Latino youth.

Salivary cortisol measures used in the paper by Barat et al. represent free cortisol, and were not associated with fat distribution (118). In fact, the salivary response to lunch was negatively correlated with trunk fat, especially in girls. This is in contrast to previous research by the same group which saw increased or similar salivary responses in obese compared with normal weight premenopausal women and obese compared to non-obese children (118).

These two studies indicate that there is likely a relationship between circulating cortisol levels and adiposity, particularly visceral (abdominal) adiposity. The relationship of elevated

cortisol to insulin resistance indicated in both studies suggests that overweight and obese children with elevated cortisol may be at increased risk for T2D, and are already known to be at greater risk for MetS as demonstrated in the paper by Weigensberg.

In a 2008 paper, Soros and colleagues (119) hypothesized that some obese children may actually exhibit low cortisol levels (24-hour average), and that this is an adaptive response to the insulin resistance associated with obesity. If this hypothesis were correct, then obese children with elevated cortisol levels would be insulin resistant, as indicated by the two previous studies (117; 118) and at higher risk for development of T2D. This is an interesting hypothesis that may need to be researched further, as the mechanisms for the reduced or elevated cortisol responses are unknown (119).

Psychosocial Function and Cortisol

In adults, blunted or low-variability in cortisol patterns throughout the day are associated with adverse health effects, such as hypertension, increased abdominal deposition of adipose tissue, and low HDL cholesterol (115). Altered cortisol responses to stress have also been shown among children and adolescents, and are often associated with several psychosocial triggers (i.e. bullying) and/or effects (i.e. behavior problems) (75)(120).

Ouellet-Morin et al. (75) examined cortisol responses in a sample of 12 year-old children with a history of maltreatment. Children were twins selected from a longitudinal study cohort, and were eligible for inclusion in the sub-study if only one twin had been maltreated in the past. The Child Behavior Checklist (CBCL) was used to assess behavior problems and was completed by parents, whereas a separate assessment was completed by teachers (Teacher's Report Form). In this study, children who had been maltreated demonstrated blunted cortisol responses to a

laboratory stressor (the Psychosocial Stress Test) when compared to non-maltreated children, and these lower cortisol responses were also found to be associated with increased behavior problems and social difficulties among maltreated children only. Thus, these results suggest that early-life maltreatment is associated with altered cortisol responses to stress and also to psychosocial difficulties later in childhood.

Further research by Gustafsson et al. (74) found an inverse u-shaped relationship between cumulative stress exposure and diurnal cortisol patterns. In this study, 130 children and adolescents (mean age 12.8) were again selected from a longitudinal twin study (only one twin from each pair was included). In this study, the Coddington Life Events Scale was used to assess psychosocial function; additional information was gathered regarding lifetime exposure to chronic events (i.e. car accidents, witnessing a violent crime) and family socioeconomic status. Then, thirty-two items from these questionnaires were combined (1 SES item, 24 negative life events, 7 potentially tragic events) and counted to create a cumulative adversity “score,” such that children with greater scores had experienced greater levels of adversity. Based on these data, it was determined that adversity was significantly related to cortisol concentrations. When cortisol was examined at individual time-points, cumulative adversity was related to post-awakening cortisol (taken 30 minutes after waking) and the slope of the diurnal decline, but not to immediately upon waking or bedtime levels. Interestingly, children who had experienced moderate levels of adversity experienced higher 30-minute post-waking cortisol levels, a higher cortisol awakening response (CAR), and a steeper diurnal decline than children who had low or high adversity exposure, suggesting that some exposure to adversity causes heightened activity of the HPA axis, but that there may be a threshold past which HPA axis activity is actually reduced.

Conversely, a study by Adam et al. (73) of older adolescents found that a higher CAR was related to increased risk of developing major depressive disorder (MDD). This was true after adjusting for age, gender, health behaviors and health status, as well as various mental health measures (i.e. current and past mood and anxiety disorders), suggesting that the increased risk of MDD was likely to be related to the elevated CAR observed at baseline. However, other cortisol measures, including waking and bedtime levels, were not associated with increased risk of MDD.

In adults, a 2006 study of newly diagnosed breast cancer patients and healthy community controls (121) examined the relationship between several measures of HPA axis activity (cortisol AUC with respect to the ground, cortisol AUC with respect to the increase, early morning peak, and diurnal rhythm) and measures of psychosocial functioning. Psychosocial distress was measured using the General Health Questionnaire, which includes questions about general psychological distress and somatic complaints, similarly to the PSC used in a youth population. In this study, none of the psychosocial measures mentioned here predicted cortisol release in cancer patients or controls. Correlations did indicate a positive correlation between cAUC with respect to the increase (using morning cortisol as the baseline value) and distress among control women, but not cancer patients.

Dietary Patterns and Hypercortisolemia

Insight on the role of diet in conditions of cortisol excess can be drawn from research on Cushing's syndrome and Cushing's disease. Cushing's syndrome is a clustering of several disorders characterized by chronic glucocorticoid excess. Cushing's disease is the most common type of Cushing's syndrome, and is the result of excessive ACTH release caused by a pituitary tumor. Other types of Cushing's syndrome can result from treatment with glucocorticoid

medications, tumors in tissues other than the pituitary, and adrenal tumors. Cushing's syndrome can also be food-dependent, resulting from over-expression of glucose-dependent insulintropic polypeptide (GIP), which is characterized by glucocorticoid excess after eating. Negative-feedback inhibition of ACTH release by circulating cortisol is suppressed, ACTH is released from the pituitary without any regular pattern and the normal circadian rhythmicity in ACTH and cortisol patterns is absent in patients with Cushing's syndrome. Thus, the normal decline in cortisol levels seen overnight does not occur, and Cushing's syndrome is often diagnosed following overnight cortisol sampling or an overnight dexamethasone suppression test (82).

Hypercortisolism is present in all types of Cushing's syndrome, and chronic overexposure to cortisol results in a number of metabolic and other clinical abnormalities. Metabolic effects include obesity (particularly central fat distribution), hypertension, glucose intolerance, hyperlipidemia, and diabetes; other effects include acne, hirsutism, osteopenia, muscular weakness (especially in the lower extremities), and depression, among others (82).

Management of Cushing's syndrome generally focuses on resolution of hypercortisolism through surgical and pharmaceutical interventions, and little information is available on dietary concerns. However, a Patient Information Publication from the NIH lists a number of guidelines for Cushing's patients. These patients need to be careful to reduce sodium intake since they are prone to hypertension. Additionally, since hypercortisolism tends to lead to decreased bone mineral density, it is especially important that Cushing's patients consume adequate amounts of both calcium and vitamin D; a separate dietary guideline has not been established, but patients should be sure to consume the recommended intakes for both of these nutrients. Since high cortisol levels also predispose these individuals to insulin resistance, they should consider following a diet appropriate for individuals with diabetes, in order to maintain optimal blood

sugar and insulin levels (122). Since the DASH diet is effective in managing both hypertension and insulin resistance, as discussed in a previous section, this may be a reasonable dietary pattern for Cushing's patients to follow. Also, the DASH diet emphasizes foods that are also good sources of calcium and vitamin D (45).

Influence of Physical Activity on Stress and Cortisol

Disruptions in the relationships between food intake, PA, and stress can lead to negative consequences. In ideal circumstances, increased physical activity has positive effects on stress coping and appetite regulation. The resulting metabolic and physiological changes work to reduce the risk of chronic disease. In cases of extreme stress or poor stress coping, however, individuals may become less active and increase their intake of highly palatable "comfort foods" that tend to be very calorie dense and offer few beneficial nutrients (4).

To date, only one study has specifically examined the role of PA in the relationship between stress and obesity in youth (123). In a sample of 303 adolescents (mean age 16.6 years), Yin and colleagues assessed measures of both personal- and community-level stress and their relationship with BMI, skinfold thickness, and waist circumference. Personal stress, but not community stress, was associated with adiposity after controlling for SES, and this relationship was significantly modified by weekly days of PA. These results suggest a beneficial effect of PA on the relationship between stress and obesity, although more research is necessary to further clarify these relationships.

As previously mentioned, exercise of sufficient duration and/or intensity serves as a strong enough stressor to augment circulating cortisol levels (124). With the onset of exercise, there is an increase in circulating cortisol levels that is proportional to exercise intensity, which

peaks at a level dependent upon the duration of the exercise bout. After exercise training, cortisol levels at rest may remain unchanged or decrease slightly. Additionally, there is some evidence that the cortisol response to exercise is diminished after exercise training, such that exercise of similar intensity results in a attenuated hormonal response after training (125; 126), possibly as a result of increased clearance from the circulation. In athletes, nighttime cortisol levels are lower following daytime exercise (127), suggesting that exercise may help to lower elevated cortisol levels throughout the rest of the day.

Few papers have examined the differences in the cortisol response to exercise between normal weight and obese children. In a study of 21 lean and obese children, salivary cortisol was found to be elevated in response to exercise in obese, but not lean, children (128). However, in this same study, post-exercise cortisol was 32% lower than pre-exercise levels in obese children; the authors were not able to speculate as to the mechanism for this decrease. Exercise intensity may be a limitation in this study, as participants exercised for 26 minutes at intensity approximately equal to 60% of VO_{2max} , which may not have been high enough to elicit a response in all subjects (129). In light of increased glucocorticoid receptors and glucocorticoid sensitivity in adipose tissue, this decrease may be representative of increased cortisol uptake by the tissues.

A second study of obese boys found morning cortisol levels to be decreased following a 12-week aerobic exercise-training program. In this group, decreases in morning cortisol were accompanied by decreases in weight, leptin, insulin, and LDL-cholesterol, as well as increases in HDL-cholesterol (130). These positive changes suggest that exercise training may be instrumental in improving the MetS profile of obese children. It is important to note, however, that several methodological issues are unclear in this paper. These results are in contrast to those

of Ben Ounis and colleagues (131), who found resting cortisol levels to be significantly increased following 2 months of aerobic exercise training. The variation in results between these two studies may be explained, in part, by differences in the cortisol sampling procedure. In both studies, cortisol was measured in serum following a venous blood draw obtained in the morning (8 AM and between 7 and 8 AM, respectively) both at study enrollment and upon completion of the exercise-training program (130; 131). Although an attempt was made to control for cortisol sampling time by sampling at the same time of day, no information was given regarding the time of sampling in relationship to waking time. Because cortisol levels change rapidly in the hour following waking, even small variations in sampling with respect to waking time may result in erroneous conclusions. Due to these methodological issues and general paucity of research, additional studies are needed examining these relationships and possible mechanisms for differences between normal weight and obese individuals.

Physical Activity, Stress, and Metabolic Syndrome

Although PA and stress are each independently associated with MetS, only one study has examined their combined influence in a youth population. Holmes and colleagues assessed the modifying effects of habitual PA on the relationship between several self-reported measures of psychosocial stress and a continuous metabolic risk score (132). Among less active youth, various measures of stress (i.e. school- and sports-related self-esteem, trait anxiety) were positively associated with metabolic risk, while these associations did not exist in the high PA group. Other measures of stress, such as depression measured by the Children's Depression Inventory, were not associated with metabolic risk. These results are encouraging, because they suggest that PA can in fact modify the detrimental relationship between stress and MetS. However, the sample included boys only, and the study did not include a physiological indicator

of stress. Thus, additional studies are needed to further examine these relationships and to identify a biological mechanism for the modifying effect of PA.

Summary and Conclusions

With the alarming increase in pediatric obesity in the last several decades, conditions that were once thought of as “adult” problems are being seen more frequently in the adolescent population. It has been reported that up to 63% of adolescents possess at least one feature of MetS (133), while nearly 50% of severely obese adolescents can be diagnosed with MetS (≥ 3 features). These youth are at increased risk for CVD, T2D, and other obesity-related diseases later in life. Physical activity and diet are the most common therapeutic targets (25; 134), however these two factors do not fully account for the recent increase in obesity rates. An additional factor, garnering more attention recently, is psychosocial stress and dysregulation of the HPA axis, resulting in altered daily cortisol patterns. Increased cortisol levels, either in the morning or throughout the day, have been associated with the accumulation of visceral adipose tissue, elevated blood pressure, insulin resistance, and MetS as a whole. It is hypothesized that psychosocial stress contributes to chronically elevated cortisol, and that this increase in cortisol is the biological mechanism through which psychosocial stress is associated with obesity and MetS. Of course, it is also important to remember that obesity and MetS can exist independently of each other (i.e., an individual can exhibit some or most of the features of MetS but not be obese). Therefore, it is hypothesized that psychosocial stress contributes to chronically elevated cortisol, and that this increase in cortisol is the biological mechanism through which psychosocial stress is associated with obesity and MetS, beyond the energy balance issues accounted for by PA and diet.

Physical activity has been shown to be an effective stress-management tool, and studies in youth have shown that PA can moderate the relationship between psychosocial stress and adiposity. It is less clear, however, whether PA can alter the relationship between stress and MetS. Studies of athletes and obese youth have suggested that exercise training can lower morning cortisol levels (127; 130); however, another study in youth found that exercise training significantly increased resting cortisol levels (131). Methodological differences involving cortisol sample timing, assessment of psychosocial stress, and measurement of PA may explain some of these differences. Additionally, only one study in youth has examined the moderating effects of PA on the relationship between stress and MetS (132). Thus, additional studies are necessary to gain a better understanding of the role of PA in the management of stress and MetS in an adolescent population. Because weight loss is a long-term process, identifying the role of PA in the management of stress and MetS can be used to design interventions that maximize the stress-management benefits of PA. The result may be short-term health improvements, even in the absence of weight-loss.

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CHAPTER 3: METHODOLOGY

Specific Aims and Hypotheses

Specific aim 1: Determine the relationship between daily cortisol levels and the continuous metabolic syndrome score (cMetS) in obese adolescents. It was hypothesized that cortisol area under the curve (cAUC) would be positively related to cMetS.

Specific aim 2: Determine if habitual physical activity (MVPA and/or steps/day) attenuates the relationship between daily cortisol and cMetS in obese adolescents. It was hypothesized that there would be a significant interaction of PA and cAUC, such that PA would attenuate the relationship between cAUC and cMetS.

Specific Aim #3: Determine the relationship between psychosocial functioning score and cAUC in obese adolescents. It was hypothesized that psychosocial functioning would be positively related to cAUC.

Specific Aim #4: Determine the relationship between psychosocial functioning score and cMetS in obese adolescents. It was hypothesized that psychosocial functioning would be positively related to cMetS.

Specific aim 5: Determine if habitual physical activity (MVPA and/or steps/day) attenuates the relationship between psychosocial functioning score and cMetS in obese adolescents. It was hypothesized that there would be a significant interaction of PA and psychosocial functioning, such that PA would attenuate the relationship between psychosocial functioning and cMetS.

Methods

Study design and subjects.

The study design was cross-sectional. Subjects between the ages of 12-18 years were recruited from The Healthy Weight Center (HWC; a pediatric obesity clinic) and Academic General Pediatrics (AGP) clinic at Helen DeVos Children's Hospital in Grand Rapids, MI. Patient charts were screened by the investigator for body mass index (BMI), and those with a BMI $\geq 95^{\text{th}}$ age- and sex-specific percentile of CDC growth chart were identified for recruitment. Eligible patients were approached by the investigator at the time of their clinic visit and invited to participate at that time. A sample size of 50 adolescents was included due to budgetary constraints (see power analysis at the end of this chapter). Each subject gave assent and parental informed consent was obtained before study participation. The study protocol was approved by Institutional Review Boards (IRB) at Michigan State University and Spectrum Health. Data collection was completed between January and June of 2012.

Assessment of the main outcome – metabolic syndrome.

Height and body mass were measured according to standard procedures (1). Height was measured in duplicate to the nearest 0.1 cm using a wall-mounted stadiometer (Harpender, Great Britain). Body mass was measured in duplicate to the nearest 0.1 kg using an electronic scale (Scaltronix, White Plains, NY). Body mass index (BMI) was then calculated by dividing body mass in kg by height in m^2 and BMI percentile was determined according to the 2000 CDC growth charts (2). Waist circumference (WC) was measured to the nearest 0.1 cm at the level of the superior border of the iliac crest using a Gullick tape. Blood pressure was measured after 10 minutes of seated rest. Two were taken on the right arm with an automated sphygmomanometer

(Datascope, Accutorr Plus). Trained medical staff in each clinic obtained all physical measurements, and values were reviewed by the researcher for consistency and repeated if necessary. It was not possible for all measures to be obtained by the same person because doing so would have interrupted clinic flow at the AGP clinic. For adults, good agreement has been shown between clinic-based systolic blood pressure measures and standard measures taken by trained research assistants (ICC=0.91), while agreement between clinic-based and standard measures for diastolic blood pressure is somewhat lower (ICC=0.77)(3). Biochemical markers of MetS were determined by blood work completed routinely as part of clinic visits, including fasting measures of cholesterol (total, HDL, and LDL), triglycerides, glucose and insulin. Metabolic syndrome score was derived by regressing subject values for each MetS variable onto the age- and sex-specific 50th percentile defined by Cook and colleagues (4). Because Cook's data do not provide information regarding normative fasting blood glucose values, means and SDs reported by Jolliffe (5) were used to calculate the glucose z-scores. Resultant z-scores for triglycerides, HDL cholesterol (multiplied by -1 to account for the inverse relationship between HDL and CVD risk), waist circumference, glucose, and systolic blood pressure were then summed to produce the continuous MetS score (cMetS) as previously described (6).

Daily Cortisol Measures.

Salivary cortisol was collected during the course of one day. The daily cortisol profile has been shown to remain stable for individuals over two days of measurement (7)(8), so values from one day were expected to be a good indicator of day-to-day cortisol patterns in children. Salivary cortisol has been shown to be highly correlated with serum measures (9; 10), while mixed results have been found for day-to-day reliability, varying based on the time of day each sample was taken and the time between each measurement occasion (interclass $r=0.18-0.78$) (11). Each

subject received saliva collection supplies and detailed instructions. Subjects were asked to collect saliva samples by placing an oral swab under the tongue for one to two minutes, and then placing the swab in a storage tube. As soon as possible after collection, the subject was to place the sample in his/her home freezer until it was returned to the investigator by mail. Cortisol samples are stable unfrozen for 5-7 days, and therefore freezing and then shipping by standard mail is acceptable (12). Samples were collected at 6 time points: 1) immediately upon waking, 2) 30 minutes after waking, and 3) four additional samples taken at three-hour intervals synchronized to waking time (e.g., 7:00 AM, 7:30 AM, 10:00 AM, 1:00 PM, 4:00 PM, and 7:00 PM). A graphical representation of sampling times is shown in Figure 3.1. Subjects were instructed to avoid eating a major meal 60 minutes before sample collection, and to thoroughly rinse the mouth 10 minutes prior to collection. Each tube was pre-labeled with a sample number, and subjects were directed to record the time of sampling on a paper log.

To ensure compliance with the sample collection protocol, the investigator communicated with subjects at specific times on the sampling day via text message and/or telephone. If the family desired, the investigator called the subject night before sample collection to inquire about his or her planned waking time and to remind the subject to take the first sample immediately upon waking. The following morning, the investigator sent a text message to the subject or called the specified phone number at the pre-arranged waking time. This message served as a reminder to collect the sample, as well as an opportunity to verify that the sample was collected at the appropriate time. If the sample was not collected at the correct time, the investigator clarified that the subject knew when the next sample should be taken, and the incorrect sample was not included in the analysis. Each collection tube was be labeled with a code (i.e. blue circle), which the subject uncovered at the time of collection. The subject was

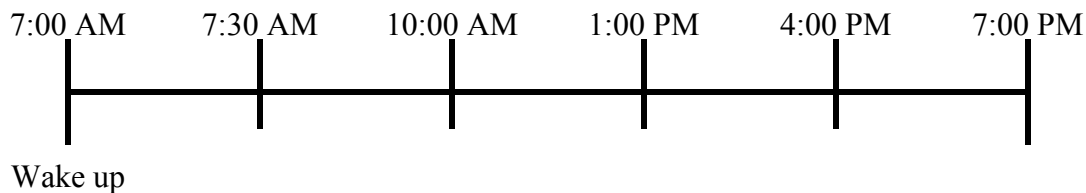
directed to reply to the investigator's text message or call with the appropriate code and time of sample collection. For each sample that was collected at the appropriate time, the investigator added \$1.00 to the subject's final incentive amount. Previous studies that have employed similar sampling protocols have included little data regarding compliance. Oskis et al. followed a similar text message reminder protocol and reported that all subjects responded to the wake-time reminder message, but did not provide any additional information regarding the number of participants who completed all saliva collection (8). Kudielka et al. used a very similar sampling protocol (samples at waking, 30-minutes post-waking, 11 AM, 3 PM, 8 PM, and 10 PM). In their study, 74% complied with sampling instructions (monitored via an electronic device) while 26% failed to take at least one sample at the correct time, and most who missed one sample also missed at least one more (13).

Once received, samples were stored at -80°C in a secure freezer at Spectrum Health. Upon the completion of data collection, all samples were transferred on ice to a laboratory at Michigan State University for analysis. Upon arrival at the laboratory, samples were allowed to warm to room temperature, centrifuged for 15 minutes at 3,000 RPM, and clear saliva was separated from the precipitants. Samples were analyzed in duplicate by enzyme-linked immunoassay (ELISA) using a high sensitivity salivary cortisol kit (Salimetrics; State College, PA). Subjects were required to collect a minimum of 3 samples (immediately upon waking, 30-minutes after waking, and the final evening time point) to be included in the analysis. Cortisol AUC was calculated according to the following formula:

$$AUC = 0.5\left(\frac{T1+T2}{2}\right) + 2.5\left(\frac{T2+T3}{2}\right) + 3\left(\frac{T3+T4}{2}\right) + 3\left(\frac{T4+T5}{2}\right) + 3\left(\frac{T5+T6}{2}\right)$$

where T1=cortisol sample 1, T2=cortisol sample 2, T3=cortisol sample 3, T4=cortisol sample 4, T5=cortisol sample 5, and T6=cortisol sample 6.

Figure 3.1. Example timeline for saliva sampling.



Habitual physical activity.

Subjects were asked to wear the SenseWear Pro III armband (SWA, Body Media, Pittsburgh, PA) for 7 consecutive days to assess habitual physical activity. The SWA has been shown to be valid in healthy adults (14), children (15–17), and obese adults (18) and children (19). The armband is worn over the right triceps and uses sensors to measure galvanic skin response, skin temperature, heat flux, and near-body temperature; a 2-axis accelerometer is also contained in the unit to measure movement (17). Additionally, subjects were asked to wear the SWA to bed on two nights during the data collection period (one the night before saliva sample collection). Following the 7-day period, activity data were downloaded and analyzed for habitual physical activity (total energy expenditure; time spent in moderate-, and vigorous PA, and steps/day) using version 7.0 of the manufacturer's software.

In order to be included in the PA analysis, subjects were required to have at least 4 days (3 weekdays and one weekend day) of data, each day including at least 10 hours of wear time during waking hours. To insure compliance, the minimum standard of armband wear was included in the requirements to be eligible for study incentives.

Psychosocial Functioning

Psychosocial functioning was evaluated using the Pediatric Symptoms Checklist (PSC) (20). The PSC is a 35-item questionnaire designed for use by pediatric practitioners to detect psychosocial problems among their patients. For each item, the child indicated whether the statement describes the child never, sometimes, or often. Responses coded as “never” received a score of 0 points, “sometimes” received a score of 1 point, and “often” received a score of 2 points, and these scores were summed to compute a total score. Recent factor analyses completed with healthy children in pediatric primary care identified subscales indicative of internalizing and externalizing difficulties (21). Although these factor analyses were completed using the parent report form of the PSC, the developers of the questionnaire communicated that the scales should be applicable to the youth report, as well (personal communication). For the full PSC, as well as the internalizing and externalizing sub-scores, a higher score is indicative of greater psychological dysfunction. For the youth report of the PSC, a total score ≥ 30 is said to indicate impaired psychosocial functioning. For the purposes of this study, the total score on the PSC was used as an indicator of psychosocial function.

Pubertal Stage

Pubertal stage was physician-assessed at the time of the clinic visit (first visit at the HWC/well child visit at AGP). Physicians use Tanner’s pubic hair, breast, and genital criteria (22). Of these, pubic hair is generally considered to be the most accurate criterion for use with obese adolescents (23). Pubertal staging was used as a covariate in data analysis.

Dietary assessment.

Habitual dietary patterns were assessed using a 3-day diet record, which is included in standard assessments at the HWC; subjects recruited from AGP were given detailed instructions and asked to record all of their daily food intake on 3 days (2 days of their choice and the day of cortisol sampling) during the week-long physical activity monitoring period. Subjects from AGP were asked to return the diet records with their samples and SWA, while diet records for HWC patients were retrieved from their medical record. Diet data were then analyzed and the healthy eating index (HEI) was calculated according to Guenther et al. (24). In short, 10 components are included in the HEI (the five major food groups, total dietary fat, saturated fat intake, cholesterol intake, sodium intake, and dietary variety); each of these 10 components is scored on a range from 0-10, so that subjects receive a diet quality score ranging from 0-100, with a higher score indicating superior dietary quality. Scores for the major food groups are based on the age- and sex-specific total daily serving recommendations for each individual. The two components dealing with fat intake, components 6 and 7, are scored based on daily recommendations for each nutrient, such that individuals consuming less than the recommended daily intake receive a score of 10. Components 8 and 9, cholesterol and sodium, are based on milligrams consumed per day. Finally, dietary variety is assessed by counting foods consumed at a minimum of one-half serving in each food group and equivalents are added to determine how many different foods each individual consumed. A perfect score of 10 is assigned to this category if an individual consumes at least 16 different foods over the 3-day study period. The HEI was included to be used as a covariate in cortisol analyses.

Data analysis.

Descriptive statistics were computed for physical characteristics and individual components of MetS, habitual physical activity, psychosocial stress score (obtained via the PSC),

as well as cortisol AUC and at each individual time point. For continuous variables, differences between boys and girls and between subjects recruited from the HWC and AGP clinics were evaluated using an independent samples t-test. Differences in categorical variables between boys and girls and between subjects recruited from HWC and AGP were evaluated using chi-square analysis.

Specific aim 1: Determine the relationship between daily cortisol levels and the continuous metabolic syndrome score (cMetS) in obese adolescents.

Linear regression, with and without controlling for pubertal stage, age and sex, was used to examine the association between cortisol AUC and cMetS. Additionally, partial correlation, again controlling for pubertal stage, age, and sex, was used to examine the relationship between cortisol at specific time points (waking, 30-minutes post-waking, and 12-hours post-waking) and individual components of MetS (lipids, blood pressure, waist circumference and BMI, glucose, and insulin).

Specific aim 2: Determine if habitual physical activity (MVPA and/or steps/day) attenuates the relationship between daily cortisol and cMetS in obese adolescents.

Possible effect modification of PA level was determined using multiple regression. Predictors entered into the model included cortisol AUC and MVPA (min/day). The interaction between cAUC and MVPA was examined first, with and without controlling for pubertal stage, sex, and age. This technique was then repeated in order to examine the interaction between cAUC and steps/day on cMetS. Differences in the resultant regression equations from aim 1 and aim 2 were examined using an F test.

Specific Aim #3: Determine the relationship between psychosocial functioning score and cAUC in obese adolescents.

Partial correlation, controlling for pubertal stage, age, and sex, was used to examine the relationship between psychosocial function score (PSC total score, internalizing score, and externalizing score) and cortisol AUC. The same technique was used to examine the relationship between psychosocial function and cortisol at specific time points (i.e. waking, 30-minutes post-waking), for the total score and internalizing and externalizing sub-scores.

Specific Aim #4: Determine the relationship between psychosocial functioning score and cMetS in obese adolescents.

Linear regression, with and without controlling for pubertal stage, sex, and age was performed to examine the relationship between psychosocial function score and cMetS. Additionally, partial correlation, again controlling for pubertal stage, age, and sex, was used to examine the relationship between PSC scores (total, internalizing, and externalizing) and individual components of MetS.

Specific aim 5: Determine if habitual physical activity (MVPA and/or steps/day) attenuates the relationship between psychosocial functioning and cMetS in obese adolescents.

Possible effect modification of PA level was determined using multiple regression. Predictors entered into the model included total score from the PSC and MVPA (min/day). The interaction between PSC score and MVPA was examined first, with and without controlling for pubertal stage, sex, and age. This procedure was then repeated using PSC total score and

steps/day as predictors Differences in the resultant regression equations from aim 4 and aim 5 were examined using an F test.

An alpha level of 0.05 was used for statistical significance. A priori power analyses identified a projected sample size of 68 adolescents in order to obtain a statistical power level of 0.80 with a Type I error rate of 5% ($\alpha=0.05$) and a medium effect size. Due to budgetary constraints, 50 adolescents were included in this study. The expected statistical power based on this sample size was 0.76 for specific aims 1 and 2. All statistics were analyzed using SPSS version 20.0.

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CHAPTER 4: RESULTS

Subject Characteristics

Patients at the Healthy Weight Center (HWC) and Academic General Pediatrics (AGP) clinics were pre-screened for study eligibility according to the inclusion and exclusion criteria outlined previously. A total of 110 were pre-determined to be eligible based on BMI percentile and medical history free of known HPA axis disorder; 50 of these 110 agreed to participate (15 boys, 35 girls; see Figure 4.1). Of these 50 subjects, 32 completed saliva collection, 32 completed physical activity monitoring, 41 had complete glucose and lipid panels, and 38 returned a completed Pediatric Symptom Checklist (PSC). Incomplete data were distributed as follows: 11 did not return any saliva samples while 7 did not collect all required samples; 7 armbands were not returned while 11 did not have sufficient wear time; 9 did not fulfill physician orders for lab work; 12 did not return the PSC. A total of 22 had complete data for anthropometry, PA, cortisol, metabolic syndrome (MetS), and the PSC (see Table 4.1 for further explanation of the distribution of measures). There were no significant differences for anthropometry, cortisol, physical activity, or metabolic syndrome variables between those with and without complete data.

Descriptive characteristics of the sample are shown in Table 4.2. In general, boys and girls were similar in terms of anthropometric variables with boys being significantly taller in terms of absolute values, but not when expressed as age- and sex-specific percentiles. Subjects recruited from the HWC were heavier than those from the AGP clinic (105.8 ± 28.1 kg vs. 88.4 ± 21.5 kg, respectively) and had a greater BMI ($38.6 \pm$

7.2 kg/m² vs. 34.0 kg/m², respectively). In the total sample, height approximated the 49th percentile while weight approximated the 98th percentile. Eighty-eight percent of participants had a BMI \geq the 97th percentile, and 46% of BMIs exceeded the 99th percentile. The distribution of ratings of secondary sex characteristics is shown in Table 4.3. The proportion of subjects in each stage of sexual maturation did not vary between HWC and AGP clinics. It should be noted that there were not any girls classified in stage 1 for either pubic hair or breast development, which makes sense based on the mean age of girls in this study (14.5 ± 2.0 y); similarly, it stands to reason that 8-9% (n=1 to n=2) boys would be in stage 1 for either pubic hair or genital development.

Metabolic Syndrome

Mean values for the 42 subjects who completed the fasting lipid panel and glucose assessments are shown in Table 4.4. Boys and girls did not differ on any biochemical variables, waist circumference, or blood pressure. However, slight differences existed for SBP (but not DBP or MAP) between subjects from the two clinics (HWC: 123.2 ± 14.3 mmHg vs. AGP: 116.1 ± 8.1 mmHg; $p = 0.06$), as well as for WC (HWC: 122.0 ± 16.5 cm vs. AGP: 99.3 ± 13.7 cm; $p < 0.0001$). Subjects were most likely to meet MetS criteria for WC (88%), followed by HDL (64.3%) and BP (28%), while TG (26.2%) and glucose (4.8%) were least likely to be adverse (see Figure 4.2). Adolescents from the HWC were slightly more likely to have an elevated waist circumference (100% vs. 81%, respectively) and a low HDL (76% vs. 56%) than AGP adolescents, though these differences were not statistically significant ($p > 0.05$). In total, 33.3% of the sample

Figure 4.1. Recruitment flowchart.

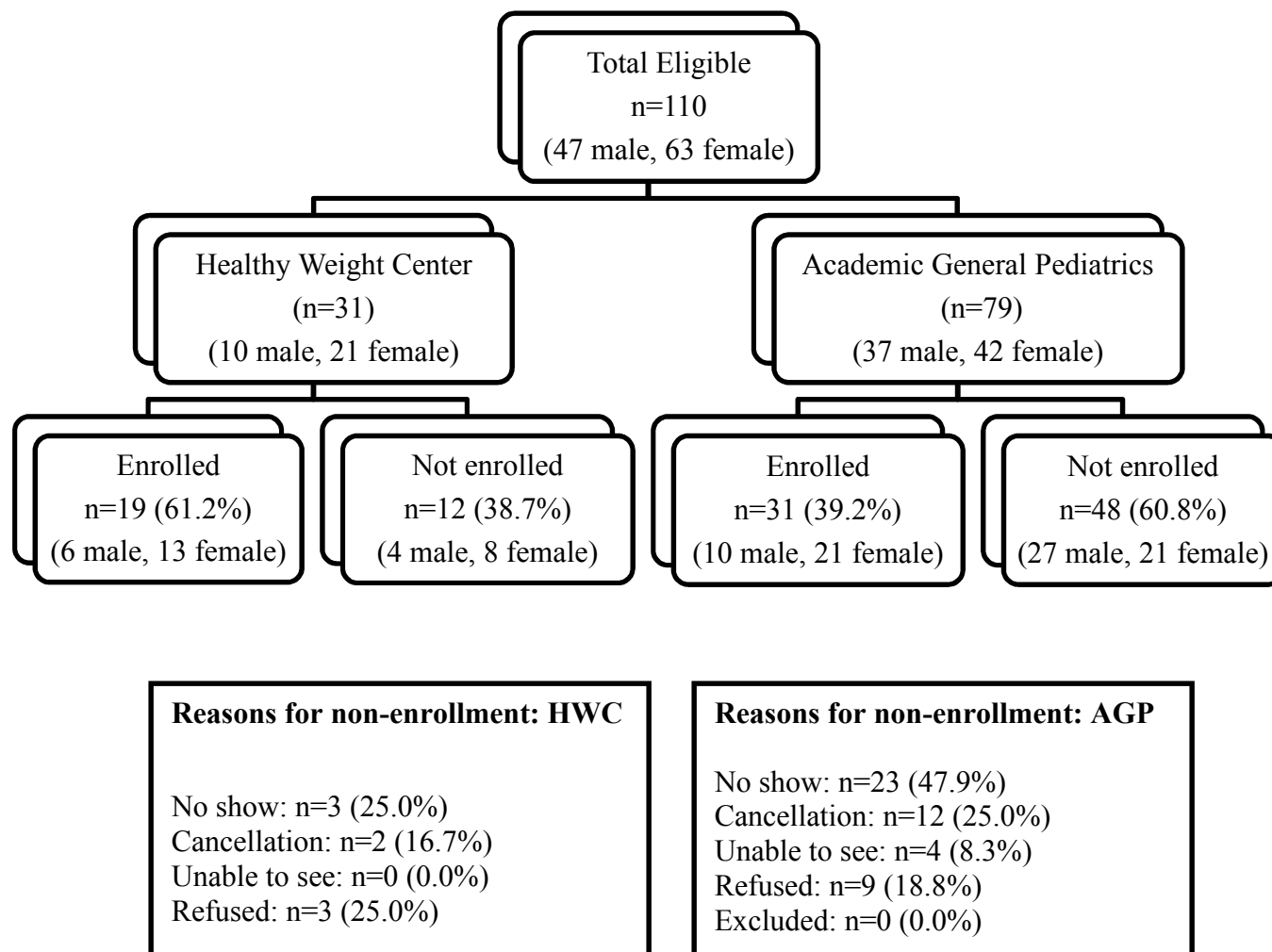


Table 4.1. Distribution of subjects who completed data collection for study variables.

	One measure	Two measures	Three measures	Four measures	Five measures
N (%)	4 (8.0)	5 (10.0)	6 (12.0)	14 (28.0)	22 (44.0)
	Anthropometry	Labs	Saliva	Physical Activity	PSC
N (%)	50 (100.0)	41 (82.0)	32 (64.0)	31 (62.0)	38 (76.0)
Anthropometry and labs		41 (82.0)			
Anthropometry, labs, and saliva			28 (56.0)		
Anthropometry, labs, saliva, PA				22 (44.0)	
All					22 (44.0)

(n=14 of 42) met the criteria for MetS. While the prevalence of MetS was slightly higher for adolescents seen at the HWC (41% vs. 28%), this difference was not statistically significant ($p>0.05$). Additionally, cMetS was positively correlated with BMI z-score ($r=0.55$, $p<0.0001$). The mean cMetS score was 4.16 ± 4.30 , and did not differ by clinic or sex.

Table 4.2. Descriptive characteristics of the sample. Values are mean (SD) unless otherwise indicated.

	Male (n=15)	Female (n=35)	Total (n=50)	Min-Max
Age (y)	15.3 (1.7)	14.5 (2.0)	14.8 (1.9)	12.3-18.9
Height (cm)	169.6 (12.1)*	158.9 (7.0)	162.1 (10.0)	142.2-186.1
Height %ile	54.5 (27.1)	47.0 (28.1)	49.3 (27.8)	6.5-97.2
Weight (kg)	105.1 (33.3)	90.7 (20.2)	95.0 (25.4)	57.2-172.0
Weight %ile	97.8 (3.3)	97.4 (3.7)	97.5 (3.6)	82.7-99.9
BMI (kg/m^2)	35.7 (7.4)	35.7 (6.9)	35.7 (7.0)	25.8-53.8
BMI percentile	98.8 (1.0)	98.2 (2.0)	98.4 (1.8)	89.7-99.9
% severe obesity (BMI $\geq 97^{\text{th}}$)	93.3%	85.7%	88%	--

* denotes statistically significant difference ($p<0.05$)

Table 4.3. Percent of subjects in each stage of pubertal development.

	Pubic Hair					Breast/Genital				
	1	2	3	4	5	1	2	3	4	5
Male (%)	8.3	16.7	33.3	20.0	13.3	9.1	9.1	36.4	27.3	18.2
Female (%)	0.0	17.9	25.0	42.9	11.4	0.0	10.0	36.7	36.7	16.7

Table 4.4. Features of the Metabolic Syndrome within the sample. Values are mean (SD) unless otherwise indicated.

	Male	Female	Total	Min-Max (n)	N
Waist circumference (cm)	110.9 (22.2)	106.6 (16.7)	107.9 (18.4)	76.5-154.1	50
Total cholesterol (mg/dl)	155.2 (26.3)	157.3 (24.8)	156.7 (24.9)	94.0-212.0	42
HDL-cholesterol (mg/dl)	42.5 (9.6)	46.3 (10.0)	45.0 (9.9)	30.0-71.0	42
Glucose (mg/dl)	112.8 (75.3)	86.2 (5.6)	93.8 (41.1)	76.0-122.0	42
Triglycerides (mg/dl)	148.8 (98.5)	112.6 (66.3)	122.9 (77.4)	37.0-394.0	42
SBP (mmHg)	121.9 (15.8)	117.5 (9.3)	118.8 (11.6)	98.0-155.7	50
DBP (mmHg)	64.5 (10.9)	67.2 (7.6)	66.4 (8.7)	47.0-86.0	50
MAP (mmHg)	83.5 (11.7)	83.4 (7.4)	83.4 (8.8)	64.0-109.2	50

Figure 4.2. Distribution of metabolic syndrome features.

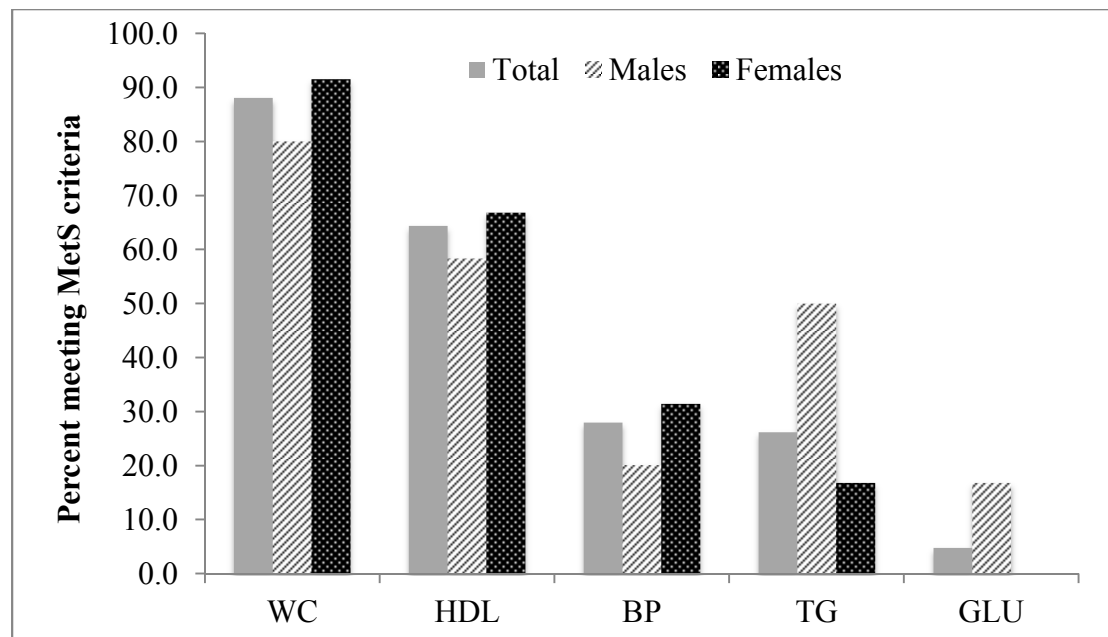


Figure legend: WC = waist circumference; HDL = high density lipoprotein cholesterol; BP = blood pressure (elevated SBP and/or elevated DBP); TG = triglyceride; GLU = fasting blood glucose (the prevalence of adverse GLU in females was 0.0%). Prevalence of MetS = 33.3% (males = 41.7%, females = 30.0%)

Habitual Physical Activity

Of the 50 subjects, 31 (62%) met the minimum wear-time criteria of 4 days for at least 10 h/day. Mean values for MVPA, steps per day, and TDEE are shown in Table 4.5. Subjects recruited from AGP were more active than subjects from the HWC when measured by steps/day, but no statistically significant differences were found between the sexes or clinics for MVPA or TDEE. Subjects with and without MetS were similar in terms of TDEE and MVPA; however, subjects without MetS took significantly more steps per day than those with MetS (9460 ± 2880 steps/day vs. 7053 ± 2637 steps/day, respectively).

Table 4.5. Physical activity and total daily energy expenditure within the sample. Values are mean (SD) unless otherwise noted.

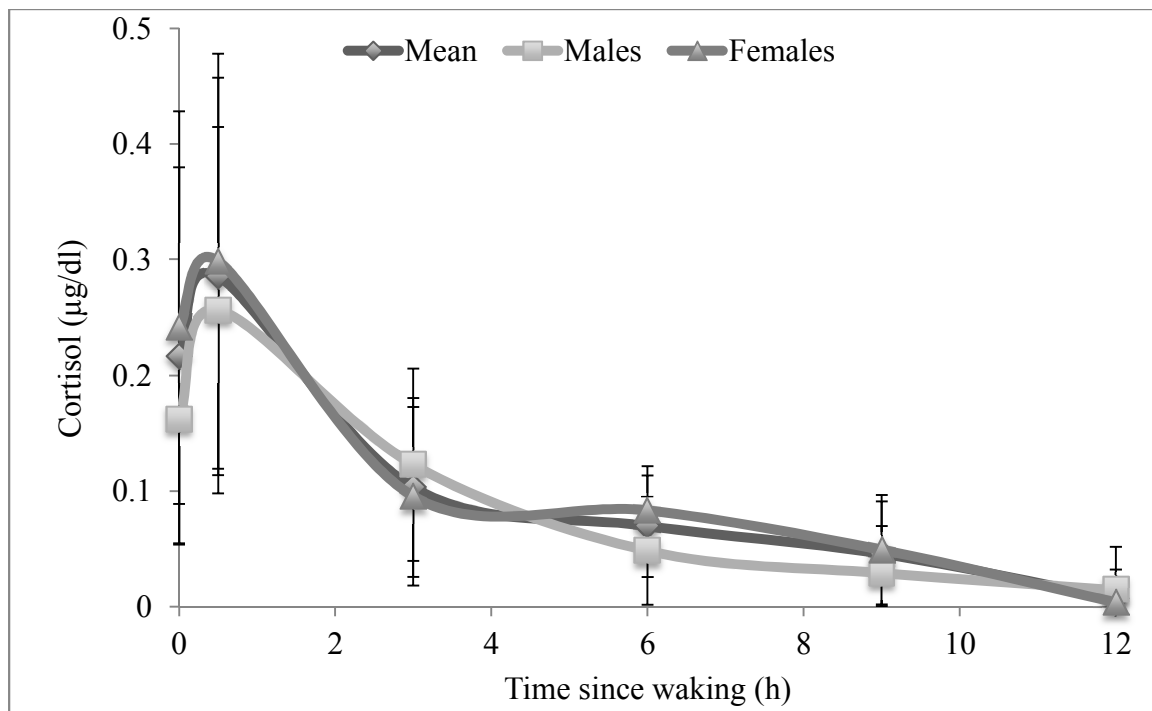
	Male (n=8)	Female (n=24)	Total (n=32)	Min-Max
MVPA (min/day)	54.0 (22.4)	43.6 (26.2)	46.2 (25.3)	4.5-109.0
Steps/day	7774 (2479)	8673 (3223)	8441 (3035)	2858-16,010
TDEE (kcal/day)	3365.7 (858.8)	2766.8 (714.0)	2921.4 (785.4)	1876.1-4956.0
TDEE (kcal/kg/day)	30.1 (4.8)	29.8 (6.8)	29.9 (6.3)	19.1-54.0

Cortisol

A total of 39 subjects returned saliva samples, and 32 (64%) of the 50 subjects met the criteria for inclusion in the cortisol analyses. Of these 32 subjects, 5 correctly obtained six samples, 9 correctly obtained five, 10 correctly obtained four, and 8 correctly obtained three. Inclusion criteria for the cortisol analysis required collection of at least the first, second, and sixth time points so that cAUC could be calculated. Figure 3 displays the mean cortisol levels at each sampling time. Mean cAUC was 1.337 ± 0.867 $\mu\text{g/dl}$ (1.180 ± 0.753 $\mu\text{g/dl}$ and $1.408 \pm$

0.922 $\mu\text{g/dl}$ for boys and girls, respectively) and did not differ by sex or clinic. Subjects with and without MetS were similar in terms of cAUC. Sixty-nine percent of the sample ($n=22$) exhibited an increase in cortisol after waking, when defined as any increase in cortisol between the waking and 30-min post sample. The intra-assay and inter-assay coefficients of variation were 2.7% and 13.8%, respectively.

Figure 4.3. Mean cortisol level at each sampling time.



Pediatric Symptom Checklist

Completed PSCs were returned by 38 (76%) of the 50 subjects. Of these subjects, 23% ($n=8$) were classified as having impaired psychosocial functioning (i.e., total score ≥ 30). There was no difference in total scores between sexes or clinics. Additionally, internalizing and externalizing scores did not differ by sex or clinic. Mean scores by sex are presented in Table 6.

The prevalence of impairment did not differ by clinic, but was slightly higher in girls than in boys (24% vs. 20%, respectively; $p = 0.50$).

Table 4.6. Pediatric Symptom Checklist. Values are mean (SD) unless otherwise noted.

	Male (n=11)	Female (n=27)	Total (n=38)	Min-Max
Total score	22.5 (11.3)	23.9 (11.2)	23.5 (11.0)	1-45
Internalizing	18.2 (7.6)	20.4 (10.2)	19.8 (9.5)	1-43
Externalizing	6.6 (2.3)	7.1 (4.3)	7.0 (3.8)	0-17
Impaired (n(%))	2 (18.2)	7 (25.9)	9 (23.7)	--

Three-Day Diet Record

A total of 38 of the 50 subjects returned diet records. Records were frequently missing serving sizes or sufficient detail to determine precisely what was eaten (i.e., “sandwich”, “noodles”), and follow-up was not completed. Therefore, it was not possible to calculate the healthy eating index (HEI) or analyze any dietary variables.

Specific Aim 1:

Specific aim 1 was to determine the relationship between the daily cortisol pattern, expressed as cAUC, and cMetS in obese adolescents. Regression analyses, first without controlling for potential covariates and second controlling for pubertal stage (pubic hair), sex, and age, are shown in Table 7. No significant relationship was found between cAUC and cMetS ($R^2=0.001$, $p=0.91$), and this relationship remained nonsignificant when pubertal stage, sex, and age were added to the model ($R^2=0.113$, $p=0.66$). Figure 4.4 illustrates the relationship between cAUC and cMetS in this sample.

Partial correlations between cAUC and individual components of MetS revealed a significant positive relationship between cAUC and insulin levels ($r=0.59$, $p=0.01$) when controlling for pubic hair stage, and sex. No significant relationships were found between cAUC and triglycerides, HDL cholesterol, waist circumference, or blood pressure measures. When examined by specific time points, cortisol sample 1 was positively related to DBP ($r=0.49$, $p=0.04$) with a trend towards significance for MAP ($r=0.43$, $p=0.07$), while cortisol sample 2 was positively correlated with insulin levels ($r=0.58$, $p=0.01$). Cortisol sample 6 was not associated with any components of MetS.

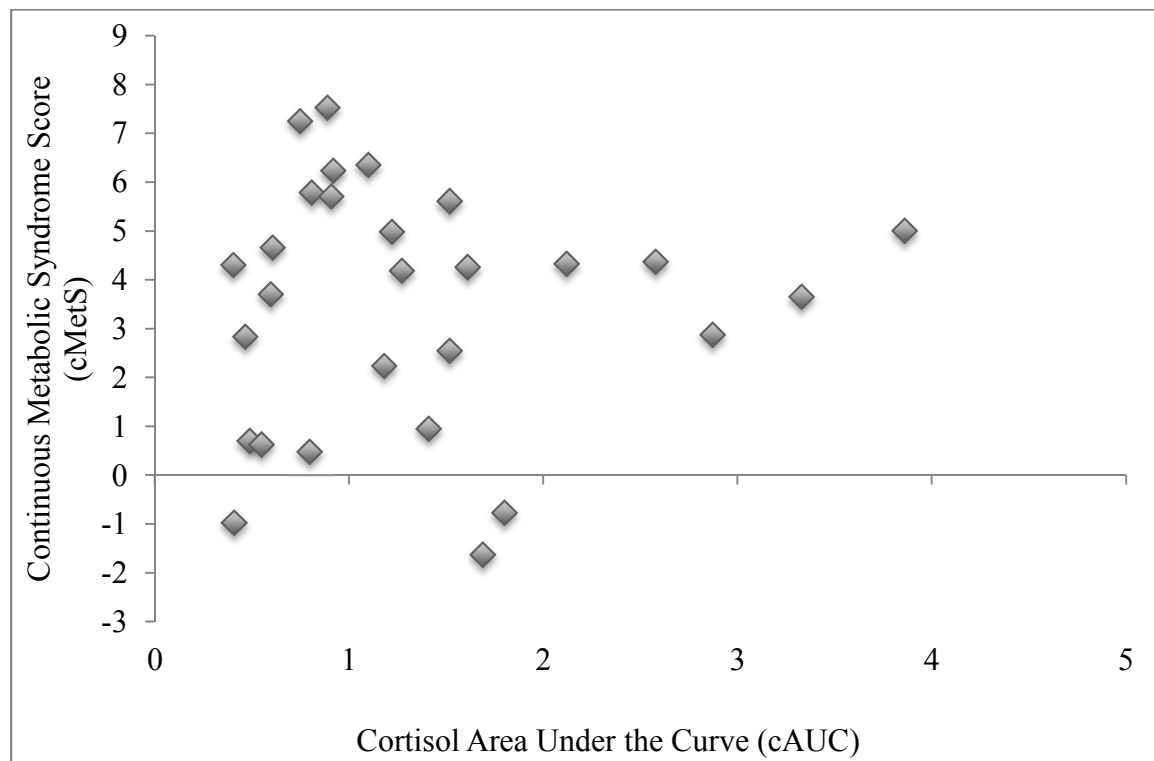
Table 4.7. Independent and combined associations of cAUC and steps/day with cMetS.

	R²	Adjusted R²	F	P
cAUC ¹	0.001	-0.045	0.013	0.91
cAUC ²	0.113	-0.074	0.606	0.66
cAUC * steps/day ¹	0.029	-0.022	0.565	0.46
cAUC * steps/day ²	0.146	-0.067	0.686	0.61
cAUC * MVPA ¹	0.045	-0.005	0.902	0.35
cAUC * MVPA ²	0.173	-0.034	0.838	0.52

¹ unadjusted model

² adjusted for age, pubertal stage (pubic hair), and sex

Figure 4.4. Relationship between cAUC and cMetS.



Specific Aim 2:

Specific aim 2 was to determine if PA attenuates the relationship between daily cortisol and cMetS, first without adjusting for potential covariates and then including pubertal stage, sex, and age as covariates. Neither the uncontrolled nor controlled models including steps/day were significant ($R^2=0.029$, $p=0.46$ and $R^2=0.146$, $p=0.61$, respectively). These regression analyses were then repeated including MVPA in the model rather than steps/day, and similar results were found (uncontrolled $R^2=0.045$, $p=0.35$; controlled $R^2=0.173$, $p=0.52$).

Specific Aim 3:

Specific aim 3 was to determine the relationship between PSC score and cAUC. Partial correlation, controlling for pubertal stage, age, and sex, indicated a significant inverse

relationship between PSC total score and cAUC ($r=-0.45$, $p=0.04$). Additional analysis of specific time points (sample 1, sample 2, and sample 6) yielded a significant inverse relationship between PSC total score and cortisol sample 2 ($r=-0.54$, $p=0.01$). No relationship was found between cortisol sample 1 or cortisol sample 6 and PSC total score. Partial correlations, again controlling for pubertal stage, age and sex, indicated a moderate inverse relationship between the PSC internalizing score and cAUC ($r=-0.48$, $p=0.03$) and cortisol sample 2 ($r=-0.57$, $p=0.01$), while there was a trend toward significance for cortisol sample 1 ($r=-0.39$, $p=0.08$). The PSC externalizing score was inversely related to cortisol sample 2 ($r=-0.50$, $p=0.02$) with a trend toward an inverse relationship with cAUC ($r=-0.43$, $p=0.05$).

Specific Aim 4:

Specific aim 4 was to determine the relationship between PSC score and cMetS. Similarly to aim 1, this was completed using regression analyses, first without controlling for potential covariates and second controlling for pubertal stage (pubic hair), sex, and age, as shown in Table 8. No significant relationship was found between PSC score and cMetS ($R^2=0.000$, $p=0.10$), and this relationship remained nonsignificant when pubertal stage, sex, and age were added to the model ($R^2=0.111$, $p=0.69$).

Table 4.8. Independent association of PSC total score and combined PSC total score + steps/day with cMetS.

	R²	Adjusted R²	F	P
PSC total ¹	0.000	-0.048	0.000	0.10
PSC total ²	0.111	-0.086	0.564	0.69
PSC total * steps/day ¹	0.049	0.003	1.077	0.31
PSC total * steps/day ²	0.164	-0.022	0.880	0.50
PSC total * MVPA ¹	0.053	0.008	1.176	0.29
PSC total * MVPA ²	0.193	0.013	1.074	0.40

¹ unadjusted model

² adjusted for age, pubertal stage (pubic hair), and sex

Specific Aim 5:

Specific aim 5 was to determine if PA attenuates the relationship between PSC score and cMetS. Results of the regression examining the interaction of PSC total score and steps/day on cMetS are shown in Table 4.8. As shown, the interaction term resulted in a stronger relationship than when the PSC total score was examined alone, however this remained non-significant (uncontrolled $R^2=0.049$, $p=0.31$ and controlled $R^2=0.164$, $p=0.49$). These regression analyses were then repeated including MVPA in the model rather than steps/day, yielding similar results (uncontrolled $R^2=0.053$, $p=0.29$; controlled $R^2=0.193$, $p=0.39$).

CHAPTER 5: DISCUSSION

The purpose of this study was to examine the association of stress (measured by survey and total daily cortisol) with metabolic syndrome in obese youth, and to evaluate whether habitual physical activity attenuated the relationship. A total of fifty adolescents agreed to participate in the study. Of these 50 adolescents, 32 completed saliva collection, 32 completed physical activity monitoring, 41 had complete glucose and lipid panels, and 38 returned a completed Pediatric Symptom Checklist (PSC)(see Table 4.1). Additional information regarding cardio-metabolic health was collected during routine clinical visits at the Healthy Weight Center and Academic General Pediatrics clinic at Helen DeVos Children's Hospital in Grand Rapids, MI. This chapter will begin by addressing primary and secondary findings and will then consider potential explanations for these results, concluding with a summary of findings.

Metabolic Syndrome

In the total sample, 33% of participants met the criteria for metabolic syndrome as outlined by Cook and colleagues (1). Previous studies of obese youth, including one from our group (2), report the prevalence of MetS in obese youth ranging from 39-53% (2; 3) - estimates vary largely as a result of the use of different cut-points and definitions of MetS described previously (see Table 2.2). Adolescents in this study were most likely to meet Cook's MetS criteria for waist circumference, followed by HDL-cholesterol, BP, and triglyceride. Adverse fasting blood glucose levels were less common, which is similar to our previous findings (2); however, in our previous report, elevated blood pressure was the least common criterion met. Clinic-measured systolic blood pressure has been shown to have good agreement ($ICC = 0.91$)

with measures taken by a trained researcher, while agreement between clinic- and researcher-measured diastolic blood pressure is slightly weaker ($ICC = 0.77$) (4).

Adolescents recruited from the HWC were more likely to meet the criteria for MetS, and to have more adverse values for each component of MetS. This was expected since the HWC sub-sample represents adolescents who are actively seeking treatment for obesity. Results are consistent with available data showing that severely obese youth experience a higher prevalence of MetS than moderately-obese youth (49.7% vs. 38.7%, respectively)(3). Although the prevalence of MetS was slightly lower in our sample, the MetS was more common in the more severely obese HWC subsample ($BMI \geq 99^{th}$ percentile) (41%) vs. the AGP subsample (28%). Although the prevalence of MetS appeared to be higher in boys (41.7%) vs. girls (30.0%), this difference was not statistically significant. Although the prevalence of MetS was expected to be higher in boys, our data demonstrate a small effect size (Cramer's $\Phi = 0.11$).

The average cMetS score in this sample was 4.16 ± 4.30 . As expected, subjects with the MetS had a significantly higher score (5.98 ± 1.60) than those without (2.38 ± 0.45 ; $p=0.004$). Metabolic risk was moderately correlated with BMI z-score ($r=0.55$, $p<0.0001$), such that more severely obese adolescents experienced higher cMetS scores. This is consistent with previous studies showing that the prevalence of MetS is higher in obese youth than their normal weight peers (3), and that heavier children tend to have higher cMetS scores (5). The mean and range of scores reported in this study are similar to those reported previously in 7-9 year-old children of all weight classifications (5). The range of cMetS scores found in this sample also supports the notion that cardiovascular risk status varies even within a cohort of “high risk,” severely-obese youth. In fact, 21% ($n=9$) of participants exhibited relatively healthy risk factor profiles, defined as meeting zero or one cut-point for MetS. Thus, it is important to remember that while obese

youth are at greater *risk* of adverse health effects, not all will experience these effects during adolescence.

Physical Activity

The SenseWear Armband (SWA) was used to assess habitual physical activity in terms of steps/day, total daily energy expenditure (TDEE), and moderate-to-vigorous physical activity (MVPA). Based on a previous validity study of v 7.0 of the SWA software, the lower cut-off for moderate-intensity PA was raised to 4.0 METs to avoid an overestimation of time spent in MVPA (6). Subjects averaged 8,434 steps per day with considerable inter-individual variation (2,858 – 16,010 steps/day). Adolescents recruited from the AGP clinic were more active in terms of steps/day than those recruited from the HWC, but no differences were found for MVPA or TDEE. Physical activity levels did not differ between boys and girls.

Based on previously published data, subjects in this study were similar in activity levels to other obese adolescents, and less active than the general adolescent population (7; 8). A recent review outlining normative step/day values for children and adolescents presented median values ranging from approximately 10,000 to 17,000 steps/day for boys and 10,000-13,000 steps/day for girls (7). Obese adolescents have been shown to take slightly fewer steps per day than non-obese youth, averaging about 10,000 steps/day in a study by Olds (8) and approximately 10,430 in a study of treatment-seeking obese children and adolescents (9). As shown in a study by Tudor-Locke (7), boys generally average higher levels of steps/day than girls; however, this was not found in the present study. Daily step counts between boys and girls in the current study varied slightly, with girls taking more steps/day than boys, which is in contrast to previous studies showing the boys generally take more steps than girls. This discrepancy may be explained by our uneven and small sample size, as only 8 boys had complete

physical activity data compared to 24 girls; however, the observed effect size was small (Cohen's $d = 0.11$). The difference in steps per day seen between our study and others may also be explained by the use of different devices (i.e., SWA vs. pedometer), and the validity of steps/day as measured by the SWA has not been fully explored.

Total daily energy expenditure averaged 3365.7 ± 858.8 kcal/day in boys and 2766.8 ± 714.0 kcal/day in girls. Boys expended more calories than what has generally been shown for obese boys in a previous study when measured by doubly-labeled water (2,621 kcal/day) and earlier versions of the SWA software [vs. 5.1 = 2,577 kcal/day, and v. 6.1 = 2,112 kcal/day, each for the total sample]; however, values for girls were similar to those previously reported by doubly-labeled water (2,540 kcal/day)(10). Absolute values reported in the current study for the total sample are also in excess of those reported in normal weight 9-16 and 10-14 year-old children and adolescents (8; 11), as well as overweight and obese 9-16 year-olds (8). However, when expressed as a relative value per kg of body weight, the values seen in this study (30.1 ± 4.8 kcal/kg/day and 29.8 ± 6.8 kcal/kg/day for boys and girls, respectively) are less than those reported in normal weight youth. Although not reported directly in the paper, values published by Dorminy and colleagues (11) based on whole-room calorimetry indicated relative TDEE of 43.2 kcal/kg/day for the total sample (it is not possible to calculate values for boys and girls based on the data reported), which is 144% greater than the relative energy expenditure seen in the current study (29.9 ± 6.3 kcal/kg/day for the total sample). Additionally, DLW values from overweight and obese youth published by Backlund and colleagues (10) indicated relative TDEE of 50.7 kcal/kg/day for the total sample, 48.2 kcal/kg/day for girls, and 57.0 kcal/kg/day for boys. This discrepancy between the Backlund sample and the normal weight sample of Dorminy is probably explained by differences in lean body mass; however, body composition data are not

available for either the current study or the Backlund study, so it is not possible to compare body composition between the two.

Mean values of MVPA found in this study are similar to those reported elsewhere for both normal weight and obese youth. Trost and colleagues (12) reported objectively-measured (accelerometry) physical activity levels of approximately 70 min/day in obese youth while Ball and colleagues (13) reported considerably lower amounts of approximately 43 min/day for obese girls and 38 min/day for obese boys (ages 13-17 y). Studies reporting objectively measured MVPA in normal weight youth or samples not divided according to weight status tend describe higher values, including mean values of approximately 60 min/day (14; 15), 80 min/day (16), or even 92 min/day (12). These values may vary due to the use of measurement instruments and other methodological differences such as accelerometer cut-points. These values also vary dramatically from self-reported values we have previously reported in a similar sample (2)(approximately 17 min/day) and the approximately 726 min/week (104 min/day) reported in European youth participating in the HELENA study (15). Again, the lack of statistically significant sex differences in the current study may be explained by the relatively small sample of boys. However, most physical activity differences were in the expected direction, with boys participating in more MVPA and expending more kilocalories per day, although effect sizes for MVPA and TDEE were small (Cohen's $d = 0.22$ and 0.20 , respectively).

In summary, participants in the current study took fewer steps per day and had lower TDEE than normal weight youth and other obese youth reported in the literature. However, levels of MVPA reported in this study are similar to those reported elsewhere in obese youth. In this study, we raised the cut-point for moderate PA to 4 METs based on our previous validation study (6); however, time spent in MVPA may still be slightly overestimated in this sample. The

difference in steps per day seen between our study and others may be explained by the use of different devices (i.e., SWA vs. pedometer), and the validity of steps/day as measured by the SWA has not been fully explored. Additionally, PA levels of boys and girls in the current study did not differ as greatly as has been reported previously. This discrepancy between our results and others may be explained by our relatively small sample of boys. Lastly, although subjects recruited from AGP took significantly more steps/day than subjects from the HWC, no significant differences were found between clinics for MVPA or TDEE. The reason for this difference between clinics is not clear; however, HWC subjects displayed greater variability than AGP subjects in both MVPA (range: 103.1 min vs. 86.0 min, respectively) and TDEE (range: 3,080 kcal/day and 2,125 kcal/day, respectively).

Daily Cortisol

In this study, salivary cortisol, or more specifically area under the curve of daily cortisol, was used as an index of stress. Cortisol, the chief hormone of the hypothalamic-pituitary-adrenal axis (HPA axis) has attracted attention in the field of obesity and metabolic syndrome research because of the ability of the HPA axis to regulate cardiovascular, metabolic, and respiratory responses to real or perceived threats (17). The HPA axis is activated in response to long-term stressors and is often used as a biomarker of the stress response (18). When the HPA axis is functioning appropriately, cortisol is released into the circulation in a predictable pattern. Circulating cortisol levels are low just before waking, and increase quickly within the first 30-45 minutes after waking to reach their peak for the day. Following this peak, cortisol gradually decreases throughout the remainder of the day. As shown in Figure 4.3, cortisol levels in this study followed the expected patterns, with no significant differences between boys and girls at any time point. Additionally, stressors such as a meal, physical activity, mental, or psychosocial

stressors stimulate further release of cortisol and can result in alteration of the normal diurnal pattern at any time during the day (19). Because of the general predictability of this pattern, cortisol is often assessed immediately upon waking and/or 30 minutes after waking in order to assess the peak. The cortisol awakening response (CAR), or the difference between the waking and 30-min post measures, is used frequently in the psychology literature as an assessment of normal HPA axis activity (20). Cortisol area under the curve (cAUC) can also be used as an assessment of total daily cortisol exposure (21).

The dynamic nature of cortisol in the period immediately following waking and the responsivity of the HPA axis to stress pose several challenges to accurate assessment of cortisol levels. In order to accurately compare cortisol measures obtained in the morning, it is necessary to tightly control sampling time, since deviation of only a couple minutes can result in error when cortisol levels are changing quickly. Unfortunately, the best way to tightly control sampling time is via venipuncture or indwelling catheter following an overnight stay in a controlled setting (i.e. hospital or general clinical research center). Doing this provides consistent timing of sampling between subjects but induces substantial participant burden. The ability to assess cortisol levels in saliva allows subjects to collect samples at home, in free-living conditions, without the additional stress of venipuncture. However, asking subjects to collect saliva samples at home reduces the ability to tightly control sampling time. Collection of additional time points throughout the day and calculation of cAUC allows for an estimation of total cortisol exposure while still allowing for examination of individual time points, providing additional information about daily patterns.

In the current study, we attempted to control sampling time by polling subjects at the time of recruitment about their usual waking time and sending text messages and/or making reminder

phone calls at each sampling time. Additionally, each correctly taken sample was incentivized by adding one dollar to the subject's gift card reward. Subjects were required to collect at least sample 1 (immediately upon waking), sample 2 (30-min after waking), and sample 6 (12 hours after waking) in order to receive a gift card and to be included in the cortisol analysis. Of the 50 subjects, a total of 39 returned saliva samples; of these, 32 met the minimum criteria for inclusion in data analysis. The most frequently missed samples were at time points 3, 4 and 5, which is not surprising since these three samples were not incentivized as strongly as samples 1, 2, and 6. The majority of subjects (69%) experienced a cortisol awakening response (CAR) when defined as any increase over resting values. A total of 16 of the 32 subjects (50%) met the CAR threshold of at least 2.5 nmol/L that has been reported previously (22). The mean change found in the present study (4.66 ± 3.21 nmol/L) was smaller than that described in one study of healthy female adolescents (9.16 ± 7.42 nmol/L) (22), but similar to another study of male and female adolescents with and without major depressive disorder (4.77 ± 8.92 nmol/L) (23) – unfortunately, neither study provides information regarding the participants' weight status. A number of subjects ($n=10$) in the present study experienced a decrease in cortisol levels from sample 1 to sample 2. Because the percentage of non-responders in the present study is greater than other studies (22), a lack of a response and perhaps especially a decrease might be indicative of poor adherence to the study protocol. It is also possible that collecting the second sample 30 minutes after waking missed the true cortisol peak in some female subjects, which may occur as late as 45 minutes after waking (22). The variation in peak time may be related to stage of sexual maturation, as well, with post-menarcheal girls peaking later in the morning (22). However, cortisol levels at each time point, CAR, and cAUC did not vary by pubertal stage (pubic hair) in the current sample.

Cortisol increases in the post-prandial period in both lean and obese youth (24). We attempted to control for the increases in cortisol following meals by directing subjects not to eat less than 1 hour before collecting each saliva sample. Time since last meal was recorded on the sampling log filled out by each subject on the day of sampling and used to verify that subjects followed directions regarding meal timing. As seen in Figure 4.2, meals did not have much of an effect on cortisol levels in this sample.

Sexual dimorphism in cortisol levels (usually in the morning and often at school) has been reported in previous studies (25–27), but was not evident in the present study. However, previous studies that have compared boys and girls have varied in the method of cortisol measurement (saliva vs. serum), and sampling time (morning, at school, etc.). This sexual dimorphism was only evident in pubertal stage 2 in the study by Knutson et al. (26) – unfortunately, the pubertal development scale used in that study was not specified. The lack of sex differences in the present study may be related to the relatively even distribution of pubertal stages among the participants, as sex differences noted in previous studies have often matched differences according to pubertal stage (27–30). It is difficult to explain the lack of sex differences in the current study compared to others due to varying methodologies. However, the current study included a much larger sample of female subjects than male subjects, which may make it difficult to identify statistically significant differences between the sexes.

Psychosocial Functioning

In addition to the use of cortisol as a biomarker of stress, the 35-item youth report of the Pediatric Symptom Checklist (PSC) was used to evaluate overall psychosocial function. The PSC consists of 35 questions that assess symptoms and difficulties adolescents may experience during daily life, including conflicts with peers and family members, difficulties in school,

somatic complaints, attention difficulties, and negative feelings, among others. The mean PSC score in this sample was 23.5 ± 11.0 , which falls below the clinical cut-off of 30 used to identify impaired psychosocial functioning. Scores were slightly higher in HWC adolescents vs. AGP adolescents, but this difference was not statistically significant. Similarly, HWC adolescents were slightly more likely to be classified as having impaired psychosocial functioning than those from AGP, though again this difference was not statistically significant. In the total sample, 24% were classified as having impaired psychosocial functioning, which is higher than the prevalence of impairment that has been noted in previous studies in the general pediatric primary care population (31; 32). Another study of school-age children (9-14 y) assessed at primary care visits that included a large number of low SES patients found a higher rate of impairment than the two studies previously mentioned (20% vs. 8-14%)(33), but still lower than the proportion identified in the current study. We previously found similar prevalence rates in a similar treatment-seeking obese population (20.4% and 33.3%, respectively) (2; 34). Although SES was not directly assessed in the current study, approximately 50% of all patients seen at the HWC and 85% of all patients seen at AGP are eligible for Medicaid; therefore, it is reasonable to assume that the SES levels of subjects in our study are similar. Our two previous studies showing similar prevalence of psychosocial dysfunction (2; 34) were also drawn from patients at HWC and thus, SES should be similar among the three studies.

Subscales for internalizing and externalizing difficulties, identified by factor analysis of children in primary care (35), were also used to examine whether there are differences in cAUC and cMetS based on different types of psychosocial dysfunction. The mean internalizing and externalizing scores were 19.8 ± 9.5 and 7.0 ± 3.8 , respectively; mean scores were not provided in the Gardner paper, so it is not possible to compare these values to those reported previously.

Previous studies have indicated increased risk of internalizing problems (i.e. depression and anxiety) and externalizing problems (i.e. oppositional defiant disorder [ODD], attention-deficit-hyperactivity disorder [ADHD], or aggression) among overweight and obese children and adolescents than their normal weight peers (36; 37). Although treatment-seeking obese children (age 9-12 y) have been shown to score higher on internalizing and externalizing assessments than non-treatment-seeking youth (37), this is not always supported in the literature (36; 38). The present study did not show a difference between treatment-seeking (HWC) and non-treatment seeking (AGP) adolescents, although we used a different instrument than these previous studies (PSC vs. Child Behavior Checklist (CBCL)). The mechanism for potential differences between treatment-seeking and non-treatment-seeking youth is unclear, but perception of psychosocial difficulties may influence the decision to seek treatment (37).

Main Findings

Cortisol and Metabolic Syndrome

Researchers are interested in examining the relationships between cortisol and MetS primarily because of previously documented relationships between cortisol levels and insulin sensitivity in adults (39). Because cortisol is released in the body in response to stress – or perceived threats – its main purpose is to ensure availability of glucose to properly fuel body defenses (40). Increased glucose availability is achieved by decreasing responses to insulin at target tissues, inhibiting β -cell release of insulin, and increasing hepatic glucose output (41). In the present study, post-hoc data analysis shown a strong positive, correlation emerged between cAUC and insulin when controlling for gender and pubic hair stage ($r=0.59$, $p=0.01$). However, since insulin levels were only available for a portion of the subjects in this study ($n=36$), this was not included in the main analysis and HOMA-IR was not calculated. Two previous studies have

also found that cortisol levels are associated with insulin resistance among obese adolescents, and that this may also be influenced by abdominal fat (42; 43). An association between serum cortisol and changes in β -cell function and acute insulin responses to glucose has also been shown in a longitudinal study of overweight and obese Latino children and adolescents (44). Taken together, these results suggest that elevated cortisol is related to increased release of insulin in order to counteract cortisol-related insulin resistance. Over time, individuals with chronically elevated cortisol may experience declines in pancreatic β -cell functioning, eventually leading to the onset of type 2 diabetes. These results support the hypothesis posed by Soros and colleagues in 2008 (45), which suggested that decreased HPA axis activity, manifested through reduced cortisol release, may actually protect obese youth from the long-term effects of chronically elevated cortisol on insulin action and glucose tolerance.

We did not find a relationship between cAUC and blood pressure in this sample. The lack of a relationship between cortisol and blood pressure is in contrast to previous studies in adults (46) and children (43). However, a previous study from our group (47) did not support a relationship between morning cortisol and blood pressure. More recently, we have found an inverse relationship between psychosocial function score and blood pressure in obese children and adolescents (2). Tanaka and colleagues (49) also found an inverse relationship between psychosocial and somatic symptoms and blood pressure in youth, suggesting that depression-related reductions in sympathetic activity may be responsible. Another study in adults (50) found an inverse relationship between respiratory sinus arrhythmia (a marker of parasympathetic nervous system activity) and pre-ejection period (a marker of sympathetic nervous system activity) and systolic blood pressure; conversely, cortisol was not associated with blood pressure.

Mechanistically, specific stress interpretations or psychosocial dysfunction (such as depression) may be associated with stronger effects on the autonomic nervous system than the HPA axis.

When cortisol was examined at each individual time point, no significant relationships were found with MetS. Post-hoc analyses revealed a significant positive relationship between sample 1 and DBP ($r=0.49$, $p = 0.04$) and a trend towards significance for MAP ($r=0.43$, $p=0.07$). Sample 2 was positively correlated with insulin levels ($r=0.58$, $p=0.01$), but not any of the blood pressure measures. Sample 6 was not significantly related to any MetS features. The relationship between sample 2 (the early-morning peak) and insulin is consistent with those results previously reported by Barat and Weigensberg, and the relationship between sample 1 (immediately upon waking) and blood pressure is consistent with those of Weigensberg (43).

Aims 1 and 2:

The results of this study indicate that daily cortisol levels are not independently associated with metabolic syndrome in obese adolescents. The first aim of this study, to examine the relationship between cAUC and cMetS, revealed no significant relationship between the two variables. This was unexpected, as previous studies have shown cortisol levels to be related to factors of the MetS among obese youth. Weigensberg and colleagues (43) found that morning cortisol levels were positively correlated with the number of features of the MetS exhibited. However, the relationship between morning cortisol and features of MetS was no longer significant after adjusting for intra-abdominal adipose tissue (IAAT), suggesting that IAAT may lie along the causal pathway between cortisol and MetS. Although there was a wide range in absolute values in the present study, nearly all subjects met the criteria for elevated waist circumference, which has been shown to be highly related to IAAT in this population (51). However, neither IAAT nor body fat percentage were measured in the present study.

Physical activity, expressed as both steps/day and min/day of MVPA, was inversely correlated with cMetS in this sample ($r=-0.54$, $p=0.002$ and $r=-0.45$, $p=0.01$, respectively). However, there was not a significant relationship between TDEE and cMetS ($r=-0.05$, $p=0.80$). The relationships shown with steps/day and MVPA are consistent with those reported previously by the European Youth Heart Study (52), NHANES (53), AFINOS (54), and another study from Vietnam (55). However, when physical activity was added to the regression examining the relationship between cAUC and cMetS, the predictive ability of the model was improved, but failed to significantly predict variance in cMetS. This may be explained in part by our relatively small sample size, which was reduced by the failure of many subjects to collect complete data for all measures.

Conversely to the idea that IAAT lies along the causal pathway between cortisol and cMetS, results from the European Youth Heart Study (56; 57) suggest that PA influences cMetS independently of adiposity. Therefore, there must be another mechanism, not directly related to adiposity, which is responsible for this relationship. The present study was driven by the hypothesis that dysregulation of the HPA axis, even at subclinical levels, might be the mechanism responsible. To date, only one previous study has examined the role of PA in the pathway between stress and obesity in youth. In this study of 303 adolescents, personal stress was associated with adiposity after controlling for socioeconomic status, and this relationship was modified by days of PA per week (58). These results support the notion that PA is involved in the relationship between stress and obesity, however more research is necessary to clarify the mechanisms driving this relationship. Similarly, only one study has examined the combined influence of stress and PA on MetS in youth. Holmes and colleagues (16) found that, among less active youth, certain measures of stress including school- and sports-related self esteem and trait

anxiety were positively associated with cMetS, while these associations did not exist in the high PA group. These results suggest that PA modifies the relationship between stress and MetS. However, this study did not include female subjects, nor did it include a physiological measure of stress; thus, it is still not possible to identify the physiological basis for the relationships found.

Aim 3:

The PSC was included in the present study in order to link the biological markers and survey measures included in previous studies. Similar to the results for cAUC, no relationship was found between PSC scores and cMetS, yet some interesting findings emerged. A moderate inverse relationship was found between the PSC total score and cAUC ($r=-0.45$, $p=0.04$), and an inverse relationship between PSC total score and cortisol sample 2 ($r=-0.54$, $p=0.01$). These inverse relationships may further support the hypothesis (45) that cortisol release is suppressed in obese adolescents who experience chronic stress in order to protect the body from the detrimental metabolic effects of chronically elevated cortisol, especially in light of our findings linking higher cAUC levels to elevated insulin levels. It is important to note, however, that this is the first study to examine the relationships between the three PSC scores and cortisol levels, making it difficult to compare our results to those of previous studies. Therefore, these results should be interpreted with caution. Additional research is necessary to confirm the relationships between the PSC scores and cortisol levels, and to evaluate whether the PSC properly captures the types of stressors that influence adolescents' HPA axis activity. Additionally, it is unclear from our data if the PSC scores and cortisol levels are directly related, or whether a third unknown variable may be involved in this relationship.

Additional analyses revealed a moderate inverse relationship between the PSC internalizing score and cAUC ($r=-0.48$, $p=0.03$) and cortisol sample 2 ($r=-0.57$, $p=0.01$). This is consistent with the relationships between self-esteem and depression (both internalizing difficulties) and cMetS previously described by Holmes and colleagues (16). Conversely, PSC externalizing score was found to be inversely and moderately correlated with cortisol sample 2 ($r=-0.50$, $p=0.02$). A previous study of adolescent boys undergoing psychological therapy examined the relationship between cortisol samples collected immediately upon waking and in the afternoon and externalizing behavior assessed using the CBCL (59). In this study, morning cortisol levels were positively related to externalizing behavior and afternoon cortisol levels were inversely related to externalizing behavior; however, these relationships were found after treatment was complete, making comparison to the current sample inaccurate because participants were either just beginning treatment at the HWC or not receiving specific care for obesity as at AGP. The study by Schechter and colleagues also indicated that the type of stressors participants experienced was important in the relationships between cortisol and externalizing behavior, such that high daily hassles and high cortisol were related to externalizing behavior, while high exposure to stress over the lifetime in combination with low cortisol levels was associated with more externalizing behavior (59). Unfortunately, the PSC does not allow for identification of specific types of stressors experienced by the participants. Another study that examined acute responses to stress found that externalizing behavior problems were associated with a blunted cortisol response to the Psychosocial Stress Test among 12 year-old children who were bullied at younger ages or maltreated by an adult (60). While this study assessed acute cortisol responses to stress rather than basal cortisol levels, the results suggest that children with externalizing behavior problems may experience reduced

cortisol reactivity. Future studies are necessary to better understand how internalizing and externalizing problems are related to cortisol levels, both chronically and in response to acute stressors, and how this might relate to metabolic health during adolescence.

Aims 4 and 5:

Similar to aim 1, the results of this study indicated that our survey measure of stress, the PSC, was not independently associated with cMetS in obese adolescents. This was unexpected as it differs from the results of Holmes and colleagues (16), who found that specific measures of stress (i.e. self-esteem, anxiety) were associated with a sample-specific cMetS score among 8-18 year old boys. Holmes found that this relationship between stress and MetS existed only among the least active boys in the sample, while the most active boys did not exhibit this relationship. This finding is also in contrast to the results of the present study, as our regression analyses for aim 5 did not support a significant interaction effect between the PSC scores and PA for predicting cMetS in our sample. Disagreement between these two studies may be explained by several methodological differences. First, Holmes and colleagues used a number of psychological assessments that specifically addressed depression, state- and trait-anxiety, self-esteem, appearance-related teasing and perceived stress; as previously mentioned, the PSC does not allow for identification of specific psychosocial domains. Since the relationship between stress and cMetS only existed for specific domains in the Holmes study, it is possible our method was not sensitive enough to identify the same relationships. In addition, the Holmes study included boys in all weight classifications, while the current study included only obese adolescents and also included girls. This difference in the sample population, in addition to the use of a sample-specific cMetS score in the Holmes study, may explain some of the disagreement with our study.

Strengths and Limitations

This study is characterized by several strengths. Most notably, the assessment of cortisol at several time points on one day improves upon previous studies that have employed only a single waking sample or a single sample at another time of day. Although not all subjects correctly obtained six saliva samples, 32 did collect at least three samples. Previous studies have attempted to examine the relationships among a single cortisol sample obtained in the morning, either immediately upon waking or 30-45 minutes after waking, and obesity and metabolic syndrome. In contrast, daily cortisol levels may be better represented by a single sample taken later in the day, such as an early afternoon sample (21). A mid-afternoon sample was included in the present study, but unfortunately was not collected by enough subjects to be useful for analysis ($n=13$ for cortisol sample 4 and $n=12$ for cortisol sample 5). Other studies that have attempted to characterize the relationship between stress and MetS in either normal weight or overweight and obese children and adolescents have relied on survey measures of stress and thus have not been able to provide mechanistic evidence for any relationships found. The current study improves on these previous studies by including both biological and survey measures. We were also able to employ objective monitoring of physical activity as opposed to self-reported activity. Furthermore, the use of the cMetS score based on nationally-representative data is a more comprehensive measure of cardiovascular risk than previous examination of dichotomous classifications of MetS, or exclusively measures of body size and composition. To our knowledge, this is the first study to combine the cMetS score with a cumulative measure of cortisol secretion in obese youth.

The most notable limitation to the present study is compliance with data collection, especially for salivary cortisol, PA, and the PSC. Despite reminder phone calls and text messages

at the scheduled time for each saliva sample, only 32 (64%) subjects returned saliva samples that met the minimum inclusion criteria, and only 5 (10%) correctly obtained all six samples.

Samples 1, 2, and 6 were more heavily incentivized than samples 3, 4, and 5 (i.e., samples 1, 2, and 6 were required to be eligible for any compensation, whereas 3, 4, and 5 were only worth one additional dollar), and this is reflected in the fact that samples 3, 4, and 5 were more likely to be missed than the other three. Although most subjects spoke or exchanged text messages with the primary investigator at all each sampling time and filled out a sample card documenting sampling time, it is not possible to know for sure that samples were taken at the time reported. This could be improved by having a trained research assistant personally observe each subject collect each sample, or by conducting the study in a controlled environment such as a general clinical research center. However, collecting samples in such a tightly controlled environment might reduce the applicability of the results to a free-living setting and requiring a research assistant's presence for measures would be burdensome to both the researcher and participant.

Additionally, we assessed cortisol levels on a single day. Studies of day-to-day cortisol stability in adolescents are limited. However, cortisol has been shown to be moderately- to strongly-correlated on two sampling days in adolescent girls both before and after menarche (22), and in adults (21; 61). Therefore, daily variation is not expected to have a large effect on the results of the current study, but more research is necessary on day-to-day stability in adolescents' cortisol patterns.

The use of the SWA for assessment of physical activity allows for precise determination of wear time. However, several adolescents who did not agree to participate in the study expressed apprehension about wearing a monitor other kids would be able to see, or thought that it might be uncomfortable. Efforts were made to address these concerns with subjects, but it is

possible that these issues affected the amount of time subjects wore the device. As with saliva collection, requiring a minimum amount of wear time in order to be eligible for compensation incentivized SWA compliance. This could potentially be improved by more heavily incentivizing additional time, or by the use of a smaller, less noticeable and more comfortable device.

The PSC was returned by only 38 (76%) subjects. In order to avoid disruption of clinic flow, subjects recruited from AGP were given a copy of the PSC to take home and return with their samples. They were reminded to return the survey in the box with their samples and armbands; however, this was not successful in all cases. Compliance could be improved in future studies by asking subjects to complete the PSC before leaving the clinic. The use of the PSC as a pencil-and-paper assessment of psychosocial function can be seen as both a strength and a weakness, in this case. On one hand, the PSC is commonly used in primary care in order to identify children and adolescents who may experience psychosocial dysfunction and need mental health care. In this instance, the use of the PSC in this study makes our results more directly accessible to primary care physicians. Conversely, the choice of the PSC limits our ability to compare our results to those of previous studies, and precluded the examination of specific types of daily stressors and their relationship to cortisol and MetS.

Some previous studies have suggested that adolescent girls may reach a peak in salivary cortisol about 45 minutes after waking, rather than the conventionally accepted 30 minutes (22). Girls in the present study reached a slightly higher (though not statistically significant) peak than boys, so timing of peak cortisol level is not expected to be a significant limitation in this study. However, some subjects who had a relatively small CAR may have had a larger response if sampling was completed at a later time. Also, as previously mentioned, it is not possible to

know with absolute certainty how long after waking each sample was collected. Therefore, some samples may vary by a few minutes in either direction, adding uncertainty to the measurements.

Although attempts were made to control for biological maturation using secondary sex characteristics, we did not control for menstrual cycle phase or hormonal contraceptive use among post-menarcheal girls. A recent review suggests that both menstrual cycle phase and oral contraceptive use may influence the CAR (through their influence on gonadal hormones), but studies to date have been equivocal (20). Menstrual cycle phase influences plasma lipid levels in premenopausal women, such that LDL, the TC:HDL ratio, and LDL:HDL ratio are all lower during the luteal phase, when estrogen levels are higher than in the follicular phase (62). Polycystic ovarian syndrome (PCOS), characterized by insulin resistance, hirsutism, acanthosis nigricans, acne, and oligo- or amenorrhea, is reported in obese female adolescents. While PCOS is associated with insulin resistance, it has not been shown to be associated with alterations in lipid profiles when compared to girls without PCOS who were similar in terms of BMI, waist circumference, and waist:hip ratio (63), or to samples of adolescent girls of all weight classifications (64). Oral contraceptive use, however, has been associated with increased blood pressure as well as increased TC, HDL, LDL, and triglycerides (65). Therefore, it is possible that oral contraceptive use may have influenced our findings, while menstrual cycle phase probably did not.

Several dietary factors, including vegetarian diets, fiber consumption, low fat dairy consumption, are associated both with individual components of MetS and with MetS itself in adolescent samples (66–69). Moreover, obese adolescents tend to eat diets that are relatively low in many important nutrients such as mono- and poly-unsaturated fatty acids, calcium, and magnesium, and high in saturated fat and sodium, which can contribute to adverse risk factor

profiles (70–73). Additionally, while less is known about the relationship between specific nutrients and cortisol levels, patients with Cushing’s syndrome need to make efforts to reduce sodium intake and meet recommendations for Vitamin D and calcium (74). Therefore, it is important to consider dietary covariates when examining the relationships between cortisol and MetS in obese adolescents. Unfortunately, data collected via 3-day diet records were not useable in the current study because it was not possible to follow-up with subjects to clarify diary entries. Although most subjects returned diet records, very few were detailed enough to glean any information. Entries were frequently missing serving sizes or clarifying information. For instance, lunch was frequently recorded as “sandwich,” without any information regarding the type of sandwich, condiments, etc. Serving sizes were often listed as “bowl,” or “a little of everything,” or missing entirely. Therefore, we were not able to control for dietary intakes in this study. Consideration of energy balance may also be of importance in examining the relationships between cortisol and MetS in obese adolescents who are trying to lose weight. Negative energy balance results in increased cortisol levels meant to increase food intake. These increased cortisol levels can, in turn increase the desire for highly-palatable foods (75). Future studies should aim to collect quality dietary data, including total caloric intake as well as detailed macronutrient information, in order to consider the interactions of dietary factors with cortisol and MetS.

Lastly, and perhaps most importantly, this study was cross-sectional. The adolescent sample is interesting because of changes in the overall hormonal milieu, growth, and insulin sensitivity that are happening during puberty, but it is possible that earlier life influences affect the relationship between cortisol and MetS in ways that could not be assessed with this study design. On one hand, this study have been much more challenging to implement with younger

children and would require different saliva sampling techniques and more direct researcher involvement with saliva collection. On the other hand, inclusion of a younger sample would allow for the examination of relationships between cortisol and MetS without potential confounding by sex hormones, and would make it possible to follow participants through adolescence to assess change over time.

Future Directions

The results of this study offer several suggestions for further research. First, future studies should attempt to provide better control over saliva sampling times. This could be done by collecting data in a clinical setting using indwelling catheters or creating a more automated system for monitoring sampling, perhaps using a smart phone or tablet computer. Collection in a clinical setting is less ideal because an unfamiliar setting and frequent blood sampling, even with a catheter, may increase acute stress and falsely elevate cortisol levels. Saliva sampling could also be combined with 24-hour urine collection to allow for the analysis of both salivary cortisol at specific time points, and urinary metabolites. Including more frequent sampling in the morning may also allow for better determination of the morning peak, which may be helpful in the face of potential variation in peak time between the sexes (22). Finally, reliable sample collection throughout the afternoon would allow for analysis of the slope of the cortisol curve, which may be related to a number of psychosocial factors (76).

As previously discussed, the poor compliance with physical activity monitoring in the present study limits our ability to draw conclusions on the role of physical activity in the relationship between stress and metabolic syndrome. Future studies should consider alternative assessment methods, such as less conspicuous accelerometers worn at the waist, detailed physical activity diaries, or a combination of assessments.

Future studies should also consider the use of additional psychological assessments, to more closely examine specific psychosocial aspects that have been related to both cortisol and metabolic syndrome. Assessment of depression, anxiety, experiences with bullying and trauma, and familial stressors such as divorce or parental unemployment may provide a clearer picture of the effect of specific dimensions of psychosocial functioning and stressors on health in obese youth.

The results of the present study raise a number of research questions to be addressed in the future. First, the positive relationship found between cAUC and insulin levels coupled with the inverse relationships between PSC scores and cortisol levels seem to suggest the hypothesis that cortisol levels may be suppressed in a subset of obese adolescents, in an effort to protect the body from the negative effects of chronic cortisol excess (45). These relationships would be best studied using a longitudinal study design beginning in early childhood and including more comprehensive assessments of stress exposure and perception. Doing so would allow researchers to identify the timing of altered cortisol secretion, metabolic effects, and exposure/reaction to stressors. Additionally, the inclusion of markers of sympathetic and parasympathetic nervous system activity, as discussed previously, would allow researchers to consider the interactions of the different components of the autonomic nervous system for a more comprehensive assessment of the relationships between stress and metabolic health. Finally, recent research highlighting the importance of sedentary time in the development of obesity and comorbidities including psychosocial health (77) suggests that time spent being sedentary may be an important variable to include in future studies examining the role of physical activity in the relationship between stress and metabolic syndrome.

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APPENDICES

APPENDIX A

INFORMED CONSENT – HEALTHY WEIGHT CENTER

This is the informed consent document that was approved by the Spectrum Health IRB and Michigan State University IRB for participants recruited from the Healthy Weight Center.

**Permission to Take Part in a Human Research Study &
HIPAA Authorization for Release of Health Information for
Research Purposes**

Title of research study: Physical activity, stress, and metabolic syndrome in youth.

Investigator: **Emily E Hill, MA**
 Joey C Eisenmann, PhD

“You” refers you or your child.

“We” refers to Helen DeVos Children’s Hospital, Michigan State University, and the study investigators.

We invite you to take part in a research study because you have been referred to the Healthy Weight Center over concerns about your weight.

What you should know about a research study?

- Someone will explain this research study to you.
- You volunteer to be in a research study.
- Whether or not you take part is up to you.
- You can choose not to take part in the research study.
- You can agree to take part now and later change your mind.
- Whatever you decide it will not be held against you.
- Feel free to ask all the questions you want before you decide.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you talk to the investigator or members of the research team at (616) 391-8675 or (919) 593-5851.

This research has been reviewed and approved by the Spectrum Health Institutional Review Board. You may talk to them at (616) 486-2031 or irb@spectrum-health.org for any of the following:

- Your questions, concerns, or complaints are not being answered by the investigator or research team.
- You cannot reach the investigator or research team.
- You want to talk to someone besides the investigator or research team.
- You have questions about your rights as a research participant.
- You want to get information or provide input about this research.

Why are we doing this research?

We would like to find out more about daily changes in a hormone called cortisol in teenagers. Hormones help one part of the body send messages to other parts of the body. Stress (like anxiety, illness, physical activity or temperature extremes) causes the body to produce a hormone called cortisol. We will be measuring cortisol in saliva. The amount of cortisol the body produces can be related to different health problems, such as metabolic syndrome.

Several factors make up the metabolic syndrome. Blood pressure is one of these factors. Others are fats, sugar, and the hormone insulin in the blood. A mathematical formula can be used to create a metabolic syndrome score based on these factors. A higher metabolic syndrome score is related to a higher risk of health problems as people age.

We are interested in how the amount of cortisol in saliva changes at different times during the day. Also, we hope to determine if these levels change based on a person's metabolic syndrome score, physical activity and/or stress level.

How long will I be in the research?

We expect that you will be in this research study for approximately seven days.

How many people will be studied?

We expect about 50 people will be in this research study.

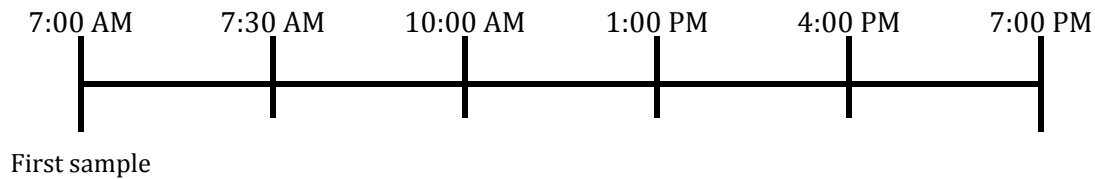
What happens if I say yes, I want to be in this research?

You have already had your initial exam at the Healthy Weight Center. In addition to what you have already been asked to do as part of your weight loss program (for example, wearing an armband to measure your physical activity), we would like for you to collect some saliva samples so that we can measure your cortisol level. We will ask you to collect a total of six samples over the course of one full weekend day.

You will be given six plastic tubes with numbered labels on them. Each tube will contain a soft, cotton-like cylinder. To collect a saliva sample, you will place one of these cylinders in your mouth for one to two minutes. Once the sample has been collected, you will put the cylinder in the plastic tube. We will ask you to write down the time each time you take a sample. We will ask you to collect six samples over the course of one weekend day, beginning when you wake up. The second sample will be taken 30 minutes after you wake up, and the remaining four samples will be taken every three-hours based on the time you collected the first saliva sample.

For example, if your first saliva collection upon waking is at 7:00 AM, you will collect samples as follows:

Figure A.1 Example timeline for collection of saliva samples.



You should not eat a meal within 1 hour before sample collection. You should also thoroughly rinse your mouth with water 10 minutes before collection. You will also be given a saliva collection log with detailed instructions. As soon as possible after collection, the samples should be placed in your home freezer. Upon completion of the seven-day period, please return the saliva samples and the armband to the researchers via mail. You will be provided with a pre-paid container for this purpose.

We would also like to use information from your medical records (for example lab values, weight, blood pressure) for this study. As part of your routine care through the Healthy Weight Center you have already agreed to wear an armband to measure physical activity over a seven-day period. The information captured through the band will be used for this study as well. All information will be kept confidential.

What happens if I say no, I do not want to be in this research?

You may decide not to take part in the research and it will not be held against you. A refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled.

What happens if I say yes, but I change my mind later?

You can agree to take part in the research now and stop at any time it will not be held against you.

Discontinuing participation will not result in penalty or loss of benefits to which you are otherwise entitled.

If you stop being in the research, already collected data will be retained in the study database. If you are participating in programs at the Healthy Weight Center, being in this study will not affect your participation in those programs.

Could being in this study be bad for me?

There are no risks involved in this study.

Will I need to pay for any of the tests or procedures in the study?

There are no costs associated with participation in this study. Procedures involved in saliva collection and analysis will not be billed to your insurance.

Will being in this study help me in any way?

There are no benefits to you from your taking part in this research. We cannot promise any benefits to others from your taking part in this research. However, possible benefits to others include gaining *a* better understanding of the relationship between physical activity and cortisol level.

What happens to the information you collect?

Efforts will be made to limit your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete confidentiality. Organizations that may inspect and copy your information for quality assurance and data analysis include:

- The Investigator and his/her research staff
- Spectrum Health staff or its agents
- The Spectrum Health Institutional Review Board (IRB) and staff
- The Michigan State University Institutional Review Board (IRB) and staff

Some of these organizations may be given direct access to your medical records for verification of the research procedures/data involved. By signing this document you are authorizing this access.

Federal law provides additional protections of your personal information. These are described in a later section.

Can I be removed from the research without my OK?

The person in charge of the research study can remove you from the research study without your approval. A possible reason for removal includes *failure to follow study procedures*.

What else do I need to know?

Payment for participating will be provided in the form of incentives awarded for completion of data collection. You will be awarded a small incentive (approximately \$1 in value), such as a code for one music download, for each requested saliva sample which was collected at the appropriate time, as determined from the saliva data collection log. Upon completion of all study data collection, you will be mailed one \$25 gift card good for use at Walmart, Meijer, or a similar store. In order to receive the \$25 gift card, you must collect at least 3 saliva samples (immediately upon waking, 30-minutes after waking, and final evening time point) and wear the armband for at least 10 hours on at least 4 days.

HIPAA Authorization for Release of Health Information for Research Purposes

The information we are asking to use and share is called Protected Health Information (PHI). It is protected by a federal law called the Privacy Rule of the Health Insurance Portability and Accountability Act (HIPAA). In general, we cannot use or share your health information for research without your permission.

What will be done with my information?

Your health information will be collected and entered in a database along with the information from other people taking part in this study.

Why am I being asked to release it?

The purpose of our study is to determine how the amount of cortisol in saliva changes at different times during the day. Also, we hope to determine if these levels change based on a person's metabolic syndrome score. We will also be measuring regular physical activity to see if this changes cortisol levels.

What will be released?

To complete this research study, we will need to collect information about you. This information may include:

- Existing medical records and medical history.
- New health information collected for purposes of this study.

Who will use it or share it?

Your information will be released by Dr. William Stratbucker. Dr. Stratbucker is the medical director of the Healthy Weight Center at Helen DeVos Children's Hospital. The researcher, Emily E Hill, MA, will receive your information. Other people may see your information. They include:

- Spectrum Health staff, employees, or other agents
- Agencies that accredit the hospital or the research program
- The study doctor and his or her staff and study personnel.
- The Spectrum Health Research and Human Rights Committee.
- The Michigan State University Institutional Review Board.

Once your protected health information has been disclosed it is possible that someone may share it again. Some people who receive your information may not be required by law to keep it confidential. We cannot guarantee that your information will not be released or made available to another party once it leaves Spectrum Health. We will share your information only if necessary. We also use all reasonable efforts to request that other individuals who receive your information protect your privacy.

How long will my health information be used?

This authorization has no expiration date.

Can I stop my protected health information from being collected?

You can cancel this authorization at any time. Your cancellation must be in writing. Once you cancel your authorization, we will stop collecting your medical information except in very limited cases if needed to comply with law, protect your safety, or make sure the research was done properly. Any information that was collected before you stopped your authorization will still be used as described above.

If you decide to stop the collection of your protected health information for this study, you must send a written notice to:

William Stratbucker, MD

Healthy Weight Center MC232

330 Barclay NE Ste 303

Grand Rapids, MI 49503

Phone: (616) 391-7999

If you have more questions about the release of your health information, you may contact the Spectrum Health HIPAA/Privacy and Information Security team at (616) 486-4113 or patient.privacy@spectrum-health.org.

What happens if I do not want you to collect and release my information?

If you decide not to authorize release of your health information as part of this study, your decision will in no way affect your medical care or cause you to lose any benefits to which you are entitled. You cannot participate in this research study if you do not authorize the use or release of your PHI.

When will it be destroyed?

Your information will be destroyed three years after study completion.

Your signature below documents your permission to take part in this research and to the use and disclosure of your protected health information. You will receive a signed copy of this complete form.

Signature of participant

Date

Printed name of participant

Signature of person obtaining consent

Date

Printed name of person obtaining consent

Your signature below documents your permission for the child named below to take part in this research and to the use and disclosure of this child's protected health information. You will receive a signed copy of this complete form.

Printed name of child

Signature of parent or guardian

Date

Printed name of parent or guardian

q Guardian (See note below)

the permission for a child only if the

Note on permission by guardians: An individual may provide permission for a child only if that individual can provide a written document indicating that he or she is legally authorized to consent to the child's general medical care. Attach the documentation to the signed document.

Signature of person obtaining consent

Date

Printed name of person obtaining consent

Assent

q Verbal Assent Obtained

q Not obtained because the capability of the child is so limited that the child cannot reasonably be consulted.

APPENDIX B:

ASSENT FOR CHILDREN – HEALTHY WEIGHT CENTER

This is the assent for children document that was approved by the Spectrum Health IRB and Michigan State University IRB for participants recruited from the Healthy Weight Center.

RESEARCH ASSENT FOR
Physical activity, stress, and metabolic syndrome in youth

Research Assent for Children Ages 12-17

Principal Investigator: Emily E Hill, MA
Joey C Eisenmann, PhD
Affiliation: Michigan State University
Helen DeVos Children's Hospital

What is a research study and why is this research study being done?

A research study is a way to learn more about something. For example, a research study might involve doctors looking at something new, such as a new medicine or new treatment to see how well it works. We would like to find out more about daily changes in a hormone called cortisol in teenagers. Hormones help one part of the body send messages to other parts of the body. Stress (like anxiety, illness, physical activity or temperature extremes) causes the body to produce a hormone called cortisol. We will be measuring cortisol in saliva. The amount of cortisol the body produces can be related to different health problems, such as metabolic syndrome.

Several factors make up the metabolic syndrome. Blood pressure is one of these factors. Others are fats, sugar, and the hormone insulin in the blood. A mathematical formula can be used to create a metabolic syndrome score based on these factors. A higher metabolic syndrome score is related to a higher risk of health problems as people age.

We are interested in how the amount of cortisol in saliva changes at different times during the day. Also, we hope to determine if these levels change based on a person's metabolic syndrome score, physical activity and/or stress level.

Why am I being asked to be in this study?

You are being asked to be in this study because you have been referred to the Healthy Weight Center over concerns about your weight.

How long will I be in the research?

We expect that you will be in this research study for approximately seven days.

How many people will be studied?

We expect about 50 people will be in this research study.

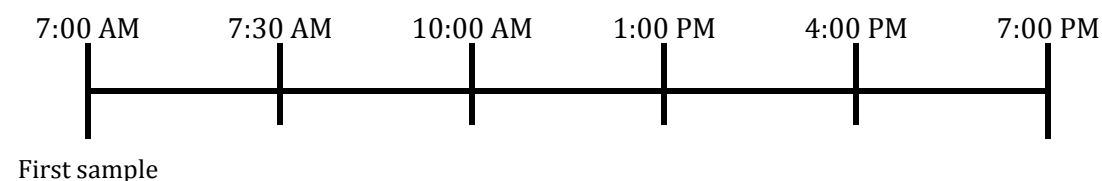
If I decide to join this research study, what will I have to do and what will happen to me?

You have already had your initial exam at the Healthy Weight Center. In addition to what you have already been asked to do as part of your weight loss program (for example, wearing an armband to measure your physical activity), we would like for you to collect some saliva samples so that we can measure your cortisol level. We will ask you to collect a total of six samples over the course of one full weekend day.

You will be given six plastic tubes with numbered labels on them. Each tube will contain a soft, cotton-like cylinder. To collect a saliva sample, you will place one of these cylinders in your mouth for one to two minutes. Once the sample has been collected, you will put the cylinder in the plastic tube. We will ask you to write down the time each time you take a sample. We will ask you to collect six samples over the course of one weekend day, beginning when you wake up. The second sample will be taken 30 minutes after you wake up, and the remaining four samples will be taken every three-hours based on the time you collected the first saliva sample.

For example, if you wake up at 7:00 AM, you will collect samples as follows:

Figure B.1 Example timeline for collection of saliva samples.



You should not eat a meal within one hour before sample collection. You should also thoroughly rinse your mouth with water 10 minutes before collection. You will also be given a saliva collection log with detailed instructions and asked to write down the time when you collect each sample. As soon as possible after collection, the samples should be placed in your home freezer. Upon completion of the seven-day period, please return the saliva samples and the armband to the researchers via mail. You will be provided with a pre-paid container for this purpose.

We would also like to use information from your medical records (for example lab values, weight, blood pressure) for this study. As part of your routine care through the Healthy Weight Center you have already agreed to wear an armband to measure physical activity over a seven-day period. The information captured through the band will be used for this study as well. All information will be kept confidential.

Will anything good happen to me if I join this study?

There are no benefits to you from your taking part in this research. We cannot promise any benefits to others from your taking part in this research. However, possible benefits to others include gaining a better understanding of the relationship between physical activity and cortisol level.

Will I be hurt if I am in the study?

There are no risks involved with this study. If you feel sick or are afraid that something is wrong with you, tell your doctor, nurse or an adult at once.

Will I be paid if I join this study?

Payment for participating will be provided in the form of incentives awarded for completion of data collection. You will be awarded a small incentive (approximately \$1 in value), such as a code for one music download, for each requested saliva sample which was collected at the appropriate time, as determined from the saliva data collection log. Upon completion of all study data collection, you will be mailed one \$25 gift card good for use at Walmart, Meijer, or a similar store. In order to receive the \$25 gift card, you must collect at least 3 saliva samples (immediately upon waking, 30-minutes after waking, and final evening time point) and wear the armband for at least 10 hours on at least 4 days.

Will people know I am in this study?

Your family and your doctor, nurse and research staff will know that you are in this study. If anyone else is given information about you, they will not know your name. A number will be used instead of your name. When we are finished with this study we might write a report about what was learned during this study. This report will not include your name or that you were in the study.

What if I do not want to be in this study?

You do not have to join this study. It is your choice. If you say yes now, you can still change your mind later. No one will be mad at you if you don't want to be in the study or if you join the study and change your mind later and stop.

Before you say **yes or no** to being in this study, we will answer any questions you have. If you join the study, you can ask questions at any time. Just tell the doctor or nurse you have a question.

I have read about this research study and my questions have been answered.

I have decided:

- ☐ **Yes, I want to be in the study**
- ☐ **No, I do not want to be in the study**

Printed Name of Child

Signature of Child (printed name only is acceptable)

Date of Assent

Printed Name of Person Obtaining Assent

Signature of Person Obtaining Assent

Date

APPENDIX C:

INFORMED CONSENT – ACADEMIC GENERAL PEDIATRICS

This is the informed consent document that was approved by the Spectrum Health IRB and Michigan State University IRB for participants recruited from Academic General Pediatrics.

**Permission to Take Part in a Human Research Study &
HIPAA Authorization for Release of Health Information for
Research Purposes
General Pediatrics**

Title of research study: Physical activity, stress, and metabolic syndrome in youth.

Investigator: **Emily E Hill, MA**
 Joey C Eisenmann, PhD

“You” refers you or your child.

“We” refers to Helen DeVos Children’s Hospital, Michigan State University, and the study investigators.

We invite you to take part in a research study because you have a BMI at or above the 95th percentile for your age.

What you should know about a research study?

- Someone will explain this research study to you.
- You volunteer to be in a research study.
- Whether or not you take part is up to you.
- You can choose not to take part in the research study.
- You can agree to take part now and later change your mind.
- Whatever you decide it will not be held against you.
- Feel free to ask all the questions you want before you decide.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you talk to the investigator or members of the research team at (616) 391-8675 or (919) 593-5851.

This research has been reviewed and approved by the Spectrum Health Institutional Review Board. You may talk to them at (616) 486-2031 or irb@spectrum-health.org for any of the following:

- Your questions, concerns, or complaints are not being answered by the investigator or research team.
- You cannot reach the investigator or research team.
- You want to talk to someone besides the investigator or research team.
- You have questions about your rights as a research participant.

- You want to get information or provide input about this research.

Why are we doing this research?

We would like to find out more about daily changes in a hormone called cortisol in teenagers. Hormones help one part of the body send messages to other parts of the body. Stress (like anxiety, illness, physical activity or temperature extremes) causes the body to produce a hormone called cortisol. We will be measuring cortisol in saliva. The amount of cortisol the body produces can be related to different health problems, such as metabolic syndrome.

Several factors make up the metabolic syndrome. Blood pressure is one of these factors. Others are fats, sugar, and the hormone insulin in the blood. A mathematical formula can be used to create a metabolic syndrome score based on these factors. A higher metabolic syndrome score is related to a higher risk of health problems as people age.

We are interested in how the amount of cortisol in saliva changes at different times during the day. Also, we hope to determine if these levels change based on a person's metabolic syndrome score, physical activity and/or stress level.

How long will I be in the research?

We expect that you will be in this research study for approximately seven days.

How many people will be studied?

We expect about 50 people will be in this research study.

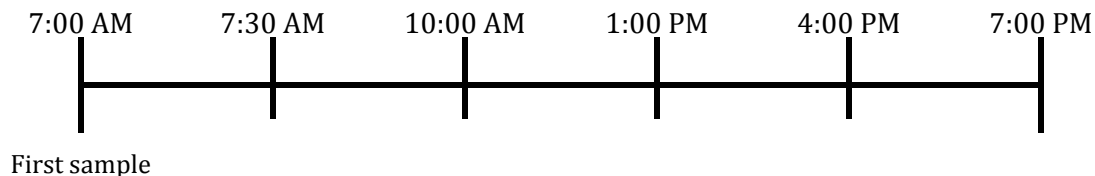
What happens if I say yes, I want to be in this research?

Some of the procedures you have already completed as part of your well child visit will also be used for this research. These include measurement of your height, weight, and blood pressure, and blood work that has been ordered by your doctor. We would also like to measure your waist circumference, which will be completed in a private exam room by one of the researchers. In addition to what you have already been asked to do, we would like for you to collect some saliva samples so that we can measure your cortisol level. We will ask you to collect a total of six samples over the course of one full weekend day.

You will be given six plastic tubes with numbered labels on them. Each tube will contain a soft, cotton-like cylinder. To collect a saliva sample, you will place one of these cylinders in your mouth for one to two minutes. Once the sample has been collected, you will put the cylinder in the plastic tube. We will ask you to write down the time each time you take a sample. We will ask you to collect six samples over the course of one weekend day, beginning when you wake up. The second sample will be taken 30 minutes after you wake up, and the remaining four samples will be taken every three-hours based on the time you collected the first saliva sample.

For example, if your first saliva collection upon waking is at 7:00 AM, you will collect samples as follows:

Figure C.1 Example timeline for collection of saliva samples.



You should not eat a meal within 1 hour before sample collection. You should also thoroughly rinse your mouth with water 10 minutes before collection. You will also be given a saliva collection log with detailed instructions. As soon as possible after collection, the samples should be placed in your home freezer. Upon completion of the seven-day period, please return the saliva samples and the armband to the researchers via mail. You will be provided with a pre-paid container for this purpose.

To measure how much physical activity you do on a normal day, we will ask you to wear an armband for one week. This armband uses sensors against the skin to measure changes in skin temperature, sweating, and heart rate. It also contains a computer chip that senses movement. The sensors are not sticky and will not prick your skin. You will be given detailed instructions for wearing the armband. A researcher will discuss these instructions with you and be available to answer any questions.

We will also ask you to complete two surveys. The first, the Pediatric Symptoms Checklist, tells us about troubles you might experience as part of your daily life. The second is a 3-day diet record. To complete this, we will ask you to write down everything you eat for 3 days.

We would also like to use information from your medical records (for example lab values, weight, blood pressure) for this study. All information will be kept confidential.

What happens if I say no, I do not want to be in this research?

You may decide not to take part in the research and it will not be held against you. A refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled.

What happens if I say yes, but I change my mind later?

You can agree to take part in the research now and stop at any time it will not be held against you.

Discontinuing participation will not result in penalty or loss of benefits to which you are otherwise entitled.

If you stop being in the research, already collected data will be retained in the study database. If you are participating in any other programs, being in this study will not affect your participation in those programs.

Could being in this study be bad for me?

There are no risks involved in this study.

Will I need to pay for any of the tests or procedures in the study?

There are no costs associated with participation in this study. Procedures involved in saliva collection and analysis will not be billed to your insurance.

Will being in this study help me in any way?

There are no benefits to you from your taking part in this research. We cannot promise any benefits to others from your taking part in this research. However, possible benefits to others include gaining *a* better understanding of the relationship between physical activity and cortisol level.

What happens to the information you collect?

Efforts will be made to limit your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete confidentiality. Organizations that may inspect and copy your information for quality assurance and data analysis include:

- The Investigator and his/her research staff
- Spectrum Health staff or its agents
- The Spectrum Health Institutional Review Board (IRB) and staff
- The Michigan State University Institutional Review Board (IRB) and staff

Some of these organizations may be given direct access to your medical records for verification of the research procedures/data involved. By signing this document you are authorizing this access.

Federal law provides additional protections of your personal information. These are described in a later section.

Can I be removed from the research without my OK?

The person in charge of the research study can remove you from the research study without your approval. A possible reason for removal includes *failure to follow study procedures*.

What else do I need to know?

Payment for participating will be provided in the form of incentives awarded for completion of data collection. You will be awarded a small incentive (approximately \$1 in value), such as a code for one music download, for each requested saliva sample which was collected at the appropriate time, as determined from the saliva data collection log. Upon completion of all study data collection, you will be mailed one \$25 gift card good for use at Walmart, Meijer, or a similar store. In order to receive the \$25 gift card, you must collect at least 3 saliva samples (immediately upon waking, 30-minutes after waking, and final evening time point) and wear the armband for at least 10 hours on at least 4 days.

HIPAA Authorization for Release of Health Information for Research Purposes

The information we are asking to use and share is called Protected Health Information (PHI). It is protected by a federal law called the Privacy Rule of the Health Insurance Portability and Accountability Act (HIPAA). In general, we cannot use or share your health information for research without your permission.

What will be done with my information?

Your health information will be collected and entered in a database along with the information from other people taking part in this study.

Why am I being asked to release it?

The purpose of our study is to determine how the amount of cortisol in saliva changes at different times during the day. Also, we hope to determine if these levels change based on a person's metabolic syndrome score. We will also be measuring regular physical activity to see if this changes cortisol levels.

What will be released?

To complete this research study, we will need to collect information about you. This information may include:

- Existing medical records and medical history.
- New health information collected for purposes of this study.

Who will use it or share it?

Your information will be released by Dr. William Stratbucker. Dr. Stratbucker is the medical director of the Healthy Weight Center and a pediatrician at Helen DeVos Children's Hospital. The researcher, Emily E Hill, MA, will receive your information. Other people may see your information. They include:

- Spectrum Health staff, employees, or other agents
- Agencies that accredit the hospital or the research program
- The study doctor and his or her staff and study personnel.
- The Spectrum Health Research and Human Rights Committee.
- The Michigan State University Institutional Review Board.

Once your protected health information has been disclosed it is possible that someone may share it again. Some people who receive your information may not be required by law to keep it confidential. We cannot guarantee that your information will not be released or made available to another party once it leaves Spectrum Health. We will share your information only if necessary. We also use all reasonable efforts to request that other individuals who receive your information protect your privacy.

How long will my health information be used?

This authorization has no expiration date.

Can I stop my protected health information from being collected?

You can cancel this authorization at any time. Your cancellation must be in writing. Once you cancel your authorization, we will stop collecting your medical information except in very limited cases if needed to comply with law, protect your safety, or make sure the research was done properly. Any information that was collected before you stopped your authorization will still be used as described above.

If you decide to stop the collection of your protected health information for this study, you must send a written notice to:

William Stratbucker, MD

Healthy Weight Center MC232
330 Barclay NE Ste 303
Grand Rapids, MI 49503

Phone: (616) 391-7999

If you have more questions about the release of your health information, you may contact the Spectrum Health HIPAA/Privacy and Information Security team at (616) 486-4113 or patient.privacy@spectrum-health.org.

What happens if I do not want you to collect and release my information?

If you decide not to authorize release of your health information as part of this study, your decision will in no way affect your medical care or cause you to lose any benefits to which you are entitled. You cannot participate in this research study if you do not authorize the use or release of your PHI.

When will it be destroyed?

Your information will be destroyed three years after study completion.

Your signature below documents your permission to take part in this research and to the use and disclosure of your protected health information. You will receive a signed copy of this complete form.

Signature of participant

Date

Printed name of participant

Signature of person obtaining consent

Date

Printed name of person obtaining consent

Your signature below documents your permission for the child named below to take part in this research and to the use and disclosure of this child's protected health information. You will receive a signed copy of this complete form.

Printed name of child

Signature of parent or guardian

Date

Printed name of parent or guardian

☐ Parent

☐ Guardian (See note below)

Note on permission by guardians: An individual may provide permission for a child only if that individual can provide a written document indicating that he or she is legally authorized to consent to the child's general medical care. Attach the documentation to the signed document.

Signature of person obtaining consent

Date

Printed name of person obtaining consent

Assent

☐ Verbal Assent Obtained

☐ Not obtained because the capability of the child is so limited that the child cannot reasonably be consulted.

APPENDIX D:

ASSENT FOR CHILDREN – ACADEMIC GENERAL PEDIATRICS

This is the assent for children document that was approved by the Spectrum Health IRB and Michigan State University IRB for participants recruited from Academic General Pediatrics.

**RESEARCH ASSENT FOR
Physical activity, stress, and metabolic syndrome in youth**

**Research Assent for Children Ages 12-17
General Pediatrics**

Principal Investigator: Emily E Hill, MA
Joey C Eisenmann, PhD
Affiliation: Michigan State University
Helen DeVos Children's Hospital

What is a research study and why is this research study being done?

A research study is a way to learn more about something. For example, a research study might involve doctors looking at something new, such as a new medicine or new treatment to see how well it works. We would like to find out more about daily changes in a hormone called cortisol in teenagers. Hormones help one part of the body send messages to other parts of the body. Stress (like anxiety, illness, physical activity or temperature extremes) causes the body to produce a hormone called cortisol. We will be measuring cortisol in saliva. The amount of cortisol the body produces can be related to different health problems, such as metabolic syndrome.

Several factors make up the metabolic syndrome. Blood pressure is one of these factors. Others are fats, sugar, and the hormone insulin in the blood. A mathematical formula can be used to create a metabolic syndrome score based on these factors. A higher metabolic syndrome score is related to a higher risk of health problems as people age.

We are interested in how the amount of cortisol in saliva changes at different times during the day. Also, we hope to determine if these levels change based on a person's metabolic syndrome score, physical activity and/or stress level.

Why am I being asked to be in this study?

You are being asked to be in this study because you have a BMI at or above the 95th percentile for your age.

How long will I be in the research?

We expect that you will be in this research study for approximately seven days.

How many people will be studied?

We expect about 50 people will be in this research study.

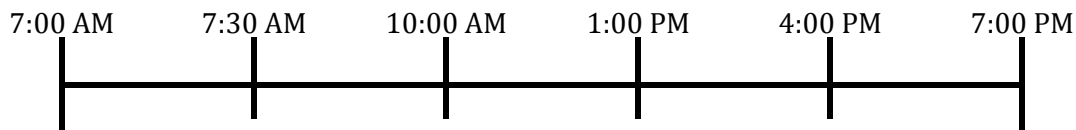
If I decide to join this research study, what will I have to do and what will happen to me?

Some of the procedures you have already completed as part of your well child visit will also be used for this research. These include measurement of your height, weight, and blood pressure, and blood work that has been ordered by your doctor. We would also like to measure your waist circumference, which will be completed in a private exam room by one of the researchers. In addition to what you have already been asked to do, we would like for you to collect some saliva samples so that we can measure your cortisol level. We will ask you to collect a total of six samples over the course of one full weekend day.

You will be given six plastic tubes with numbered labels on them. Each tube will contain a soft, cotton-like cylinder. To collect a saliva sample, you will place one of these cylinders in your mouth for one to two minutes. Once the sample has been collected, you will put the cylinder in the plastic tube. We will ask you to write down the time each time you take a sample. We will ask you to collect six samples over the course of one weekend day, beginning when you wake up. The second sample will be taken 30 minutes after you wake up, and the remaining four samples will be taken every three-hours based on the time you collected the first saliva sample.

For example, if you wake up at 7:00 AM, you will collect samples as follows:

Figure D.1 Example timeline for collection of saliva samples.



First sample

You should not eat a meal within one hour before sample collection. You should also thoroughly rinse your mouth with water 10 minutes before collection. You will also be given a saliva collection log with detailed instructions and asked to write down the time when you collect each sample. As soon as possible after collection, the samples should be placed in your home freezer. Upon completion of the seven-day period, please return the saliva samples and the armband to the researchers via mail. You will be provided with a pre-paid container for this purpose.

To measure how much physical activity you do on a normal day, we will ask you to wear an armband for one week. This armband uses sensors against the skin to measure changes in skin temperature, sweating, and heart rate. It also contains a computer chip that senses movement. The sensors are not sticky and will not prick your skin. You will be given detailed instructions for wearing the armband. A researcher will discuss these instructions with you and be available to answer any questions.

We will also ask you to complete two surveys. The first, the Pediatric Symptoms Checklist, tells us about troubles you might experience as part of your daily life. The second is a 3-day diet record. To complete this, we will ask you to write down everything you eat for 3 days.

We would also like to use information from your medical records (for example lab values, weight, blood pressure) for this study. All information will be kept confidential.

Will anything good happen to me if I join this study?

There are no benefits to you from your taking part in this research. We cannot promise any benefits to others from your taking part in this research. However, possible benefits to others include gaining a better understanding of the relationship between physical activity and cortisol level.

Will I be hurt if I am in the study?

There are no risks involved with this study. If you feel sick or are afraid that something is wrong with you, tell your doctor, nurse or an adult at once.

Will I be paid if I join this study?

Payment for participating will be provided in the form of incentives awarded for completion of data collection. You will be awarded a small incentive (approximately \$1 in value), such as a code for one music download, for each requested saliva sample which was collected at the appropriate time, as determined from the saliva data collection log. Upon completion of all study data collection, you will be mailed one \$25 gift card good for use at Walmart, Meijer, or a similar store. In order to receive the \$25 gift card, you must collect at least 3 saliva samples (immediately upon waking, 30-minutes after waking, and final evening time point) and wear the armband for at least 10 hours on at least 4 days.

Will people know I am in this study?

Your family and your doctor, nurse and research staff will know that you are in this study. If anyone else is given information about you, they will not know your name. A number will be used instead of your name. When we are finished with this study we might write a report about what was learned during this study. This report will not include your name or that you were in the study.

What if I do not want to be in this study?

You do not have to join this study. It is your choice. If you say yes now, you can still change your mind later. No one will be mad at you if you don't want to be in the study or if you join the study and change your mind later and stop.

Before you say **yes or no** to being in this study, we will answer any questions you have. If you join the study, you can ask questions at any time. Just tell the doctor or nurse you have a question.

I have read about this research study and my questions have been answered.

I have decided:

☐ **Yes, I want to be in the study**

☐ **No, I do not want to be in the study**

Printed Name of Child

Signature of Child (printed name only is acceptable)

Date of Assent

Printed Name of Person Obtaining Assent

Signature of Person Obtaining Assent

Date

APPENDIX E:

DATA COLLECTION FORM

Data Collection Form

Subject ID: _____ Date: _____
 Age: _____ Gender: _____

Inclusion Criteria:

Height _____ BMI _____
 Weight _____ BMI % _____

English speaking? ☐ Yes ☐ No
 Known disorder of HPA axis? ☐ Yes ☐ No
 Use of glucocorticoid medication? ☐ Yes ☐ No
 Eligible for study? ☐ Yes ☐ No
 Consent obtained ☐ Yes ☐ No Assent obtained ☐ Yes ☐ No

Data obtained from medical record:

	Value	Date Obtained
Lipids:		
Total cholesterol	_____	_____
HDL cholesterol	_____	_____
LDL cholesterol	_____	_____
Triglycerides	_____	_____
Glucose Tolerance:		
Fasting glucose	_____	_____
Insulin	_____	_____
Blood Pressure		
Systolic	_____	_____
Diastolic	_____	_____
Mean arterial pressure	_____	_____
Other:		
Waist circumference	_____	_____
Pubertal stage	_____	_____
PSC score	_____	_____
3-day diet record obtained	<input type="checkbox"/> Yes <input type="checkbox"/> No	

APPENDIX F:

SALIVA COLLECTION INSTRUCTIONS AND SAMPLE COLLECTION LOG

Table F.1. Instructions for collection of saliva samples.

Saliva Collection Instructions	
<p><u>Before collecting saliva, please DO NOT:</u></p> <ul style="list-style-type: none"> - Consume a major meal within 60 minutes - Consume dairy products within 30 minutes - Consume acidic or high sugar foods - Brush teeth within 3 hours - Have dental work done within 24 hours - Drink alcohol within 24 hours of collection <p><u>What to do:</u></p> <ul style="list-style-type: none"> - Rinse mouth with water - Take sample at the directed time - Put a cotton swab under tongue or roll it around across tongue. It may also be chewed slightly (Do not place it between the cheek and gum) - Make sure it's very wet - Once saturated, place cotton inside plastic insert inside the tube. - Place the sample in the freezer. 	<p><u>When to take samples:</u></p> <ol style="list-style-type: none"> 1. Right away when you wake up 2. 30-minutes after the first saliva collection 3. Three hours after the first saliva collection 4. Three hours after the 3rd sample (6 hrs after the first saliva collection) 5. Three hours after the 4th sample (9 hrs after the first saliva collection) 6. Three hours after the 5th sample (12 hrs after the first saliva collection) <p>Please use the form on the opposite side of this sheet to write down the time you take each sample, and the colored shape that is on each tube.</p>

Sampling Date: _____

Wake-up Time: _____

Figure F.1. Saliva sampling log.

Sample	Goal Time	Actual Time	Tube Code	What time did you eat last?	Comments
Example	7:00 AM	7:06 AM	Blue Square		Overslept
1					
2					
3					
4					
5					
6					

Do you have a cold or feel sick today? _____

APPENDIX G:

THREE-DAY DIET RECORD

Subject ID _____ Day _____ Date _____

Figure G.1 Three-day diet record day 1.

FOOD DAY 1

TIME	WHAT YOU ATE AND/OR DRANK	HOW MUCH? (Cup, Tablespoon, etc.)

Be as exact as possible. It is easier to record what you eat or drink right after doing so. If you wait until the end of the day, you may forget something. Be sure to record water, diet soda, gum, etc.

Subject ID _____ Day _____ Date _____

Figure G.2 Three-day diet record day 2.

FOOD DAY 2

TIME	WHAT YOU ATE AND/OR DRANK	HOW MUCH? (Cup, Tablespoon, etc.)

Be as exact as possible. It is easier to record what you eat or drink right after doing so. If you wait until the end of the day, you may forget something. Be sure to record water, diet soda, gum, etc.

Subject ID _____ Day _____ Date _____

Figure G.3 Three-day diet record day 3.

FOOD DAY 3

TIME	WHAT YOU ATE AND/OR DRANK	HOW MUCH? (Cup, Tablespoon, etc.)

Be as exact as possible. It is easier to record what you eat or drink right after doing so. If you wait until the end of the day, you may forget something. Be sure to record water, diet soda, gum, etc.

APPENDIX H:

SENSEWEAR ARMBAND INSTRUCTIONS

ARMBAND INSTRUCTIONS

Please wear the armband for one week (2 weekend days; 5 weekdays). In order to get a good measurement of your physical activity the armband needs to be worn as much as possible; 10 hours per day minimum.

Please wear the armband to bed for 2 nights while sleeping.

Be sure to take the armband off for showering or other water activities such as swimming.

After a one week period, (_____ to _____), return the armband in the mail with your saliva samples and questionnaires.

If you have questions, please call or email Emily

Phone: 919-593-5851

Email: hillemi1@msu.edu

APPENDIX I:

PEDIATRIC SYMPTOM CHECKLIST – YOUTH REPORT

Pediatric Symptom Checklist - Youth Report (Y-PSC)

Please mark under the heading that best fits you:

	Never	Sometimes	Often
1. Complain of aches or pains.....	—	—	—
2. Spend more time alone.....	—	—	—
3. Tire easily, little energy.....	—	—	—
4. Fidgety, unable to sit still.....	—	—	—
5. Have trouble with teacher.....	—	—	—
6. Less interested in school.....	—	—	—
7. Act as if driven by motor.....	—	—	—
8. Daydream too much.....	—	—	—
9. Distract easily.....	—	—	—
10. Are afraid of new situations.....	—	—	—
11. Feel sad, unhappy.....	—	—	—
12. Are irritable, angry.....	—	—	—
13. Feel hopeless.....	—	—	—
14. Have trouble concentrating.....	—	—	—
15. Less interested in friends.....	—	—	—
16. Fight with other children.....	—	—	—
17. Absent from school.	—	—	—
18. School grades dropping.	—	—	—
19. Down on yourself.....	—	—	—
20. Visit doctor with doctor finding nothing wrong.....	—	—	—
21. Have trouble sleeping.....	—	—	—
22. Worry a lot.....	—	—	—
23. Want to be with parent more than before.....	—	—	—
24. Feel that you are bad.....	—	—	—
25. Take unnecessary risks.....	—	—	—
26. Get hurt frequently.....	—	—	—
27. Seem to be having less fun.....	—	—	—
28. Act younger than children your age.....	—	—	—
29. Do not listen to rules.....	—	—	—

Pediatric Symptom Checklist – Youth Report (Continued)

Please mark under the heading that best fits you:

	Never	Sometimes	Often
30. Do not show feelings.....	—	—	—
31. Do not understand other people's feelings.....	—	—	—
32. Tease others.....	—	—	—
33. Blame others for your troubles.....	—	—	—
34. Take things that do not belong to you.....	—	—	—
35. Refuse to share.....	—	—	—