Perceived Stress, Physiological Stress, and Mental Health in Parents and Children

by

Sydney Whitney

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AUTHORS DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

ABSTRACT

Background: Elevated stress levels have been linked to a variety of health concerns, including mental disorders, making stress an important topic of research and clinical assessment. The human stress response involves the coordination of psychological and physiological processes. Psychological measures of stress, such as questionnaires, have traditionally been used to quantify stress levels, but physiological measures, such as hair cortisol concentration (HCC) are becoming increasingly popular. However, the relationship between psychological and physiological measures of stress remains unclear, and studies examining their association have yielded inconsistent results thus far.

Objectives: This thesis explored the relationship between measures of perceived stress and HCC in a clinical sample of youth with mental disorder and their parents. The specific objectives of this study were to: (1) estimate the agreement between measures of perceived stress and HCC, (2) model and compare the sociodemographic and psychosocial risk factors for elevated levels of perceived stress and HCC, and (3) model and compare the magnitude of the association between mental disorder and perceived stress and HCC, respectively.

Methods: Data came from a cross-sectional study of youth receiving mental health services and their parents. Perceived stress was measured with the Perceived Stress Scale for youth and the Parental Stress Scale for parents; physiological stress was measured with HCC. Bland-Altman analysis, limits of agreement, and intraclass correlation coefficients (ICC) were used to estimate agreement between perceived stress and HCC for youth and parents. Logistic and linear regression models were used to explore risk factors for elevated perceived stress and HCC and to compare the association between each measure of stress and mental disorder. Method of variance estimates recovery was used to compare the association between risk factors and perceived stress and HCC, respectively.

Results: Agreement between perceived stress and HCC was low in both the youth and parent subgroups (ICC = 0.31 and 0.15, respectively) and Bland-Altman plots revealed that there may be systematic differences between the measures. Several sociodemographic and psychosocial factors were found to be associated with perceived stress and HCC, however, these factors had larger measures of association with perceived stress. Higher levels of perceived stress were associated with increased odds of major depressive disorder (OR = 1.33 [90% CI 1.12, 1.57]), generalized anxiety disorder (OR = 1.10 [90% CI 1.01, 1.19]), and separation anxiety (OR = 1.14 [90% CI 1.03, 1.25]) in youth and both depression and anxiety in parents (β = 0.53 [90% CI 0.35, 0.71] and β = 0.45 [90% CI 0.26, 0.64], respectively), while HCC was associated with increased odds of generalized anxiety disorder in youth (OR = 1.14 [90% CI 1.01, 1.28]) and depression in parents (β = 0.27 [90% CI 0.06, 0.48]).

Conclusion: These findings provide preliminary evidence that agreement between measures of psychological and physiological stress is low and that each measure may have different relationships with various risk factors as well as mental disorders. This suggests that both measures should be used to comprehensively evaluate the stress

response. Additional work is needed to confirm the low agreement observed in this study and to continue to examine how psychological and physiological stress are relation to sociodemographic, psychosocial, and clinical factors.

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LIST OF ABBREVIATIONS

Autonomic Nervous System (ANS)

Hypothalamic-Pituitary-Adrenal (HPA)

Hair Cortisol Concentration (HCC)

Perceived Stress Scale (PeSS)

Parental Stress Scale (PaSS)

Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID)

Center for Epidemiologic Studies Depression Scale (CES-D)

State-Trait Anxiety Inventory (STAI)

McMaster Family Assessment Device (FAD)

Method of Variance Estimates Recovery (MOVER)

Intraclass correlation coefficient (ICC)

BACKGROUND

1.1 Stress as a concept

Stress can be broadly defined as a state of real or perceived threat to homeostasis in which environmental demands exceed an individual's resources and endanger their well-being (1–5). While stress is something that every person experiences, high levels of stress have been implicated as an important factor in the development of many diseases (6), including mental disorders (7). Because of this, stress, and particularly its connection to health, has become an important topic of research in recent years (7). However, because stress is a construct that is not directly assessable, we rely on indirect measures of the body's responses to stress to indicate its severity (8). Therefore, to evaluate the impact of stress on health, it is important to have valid and reliable methods to assess stress levels that can be used in both clinical and research settings (5).

1.2 The human stress response

The human body is usually at a point of homeostasis, where its physiological and behavioural systems are operating within optimal ranges (9). Stressors are anything that challenge this state of homeostasis (9). When stressors are encountered, they trigger a coordinated psychological and physiological response which works to improve the body's ability to withstand the stressor and return to its state of homeostasis (5,8,9).

The body's psychological response to stress encompasses an individual's perception and cognitive response to stressors (5,10). This involves both the appraisal of stressors as threats to homeostasis and a subsequent negative emotional response (2,11). Importantly, the appraisal of a stressor's threat to the individual is not determined by the stressor itself, but by a person's interpretation of the stressor in relation to their environmental demands (11). When a stressor is encountered, various cortical and limbic structures in the brain, including the amygdala, prefrontal cortex, and hippocampus, are activated (1,2,4,12). These structures are responsible for evaluating stressors, and, if the stressor is appraised as being a threat, eliciting a negative emotional response (11). This is often experienced as feelings of fear, anxiety, and worry (2,12). The cortical and limbic structures responsible for this psychological response to stressors are also closely connected to the autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis, which play important roles in the physiological response to stress (1,2). Because of this link between the structures involved in the psychological and physiological stress responses, the psychological appraisal of a stressor is thought to mediate the relationship between the stressor itself and the subsequent physiological response (5,13).

The physiological stress response follows the psychological appraisal of a stressor as a threat when the cortical and limbic structures that make this appraisal send signals to the hypothalamus (14). The hypothalamus is responsible for activating the two systems which primarily control the physiological stress response: the ANS, which provides an immediate, short-term response to a stressor, and the HPA axis, which provides a sustained response in response to chronic stress (14).

The ANS is activated by the hypothalamus, which stimulates the sympathetic nervous system and, in turn, activates the adrenal medulla, which secretes catecholamines, including epinephrine and norepinephrine (14). The release of epinephrine and norepinephrine stimulates a rapid response to stress, which can be initiated in less than a second (15), including increased heart rate, tidal volume, and stimulation of skeletal muscles (14). These changes allow an individual to quickly respond to a perceived threat, however, the output of epinephrine and norepinephrine from the adrenal medulla is limited and therefore this immediate stress response subsides quickly (14). Therefore, if the stressor is still present, additional physiological systems must be activated to maintain the stress response in the presence of chronic stress (14).

In humans, the HPA axis is also activated after a stressor is appraised as a threat, but its activation is slower than the ANS, peaking a few minutes after the onset of the stressor (16,17). Activation of the HPA axis occurs when the paraventricular nucleus of the hypothalamus secretes corticotropin-releasing hormone (7,10,18). Corticotropin-releasing hormone then stimulates the anterior pituitary gland to release adrenocorticotropic hormone (7,10,18). Adrenocorticotropic hormone is carried to the adrenal glands, which secrete glucocorticoids, including cortisol from the zona fasciculata of the adrenal cortex (7,10,18). Cortisol helps to maintain the body's natural homeostasis through the regulation of fat and glucose metabolism, blood pressure, and inflammatory and immune responses (18–20). Under stressful conditions, when the body's homeostatic balance is disrupted, increased levels of cortisol are released which induce physiological and behavioural changes to help the body cope with the stressor (19). These changes are adaptive and designed to redirect energy supplies to increase the individual's chances of survival (2). The adaptative responses range from physical changes such as increased cardiovascular tone, respiratory rate, suppression of the immune system, and blood glucose and fat concentrations to behavioural changes such as increased arousal and alertness, focused attention, and improved cognition (2). Unlike the ANS, the secretion of cortisol and subsequent physical and behavioural changes can be maintained for longer periods of time (14,17). This allows for a continued physiological response to stress if the psychological response continues to appraise the stressor as a threat (14).

Normally, the release of cortisol is regulated by both positive and negative feedback loops which ensure cortisol production stays within certain limits, depending on current stress exposure (9,10). For example, adrenocorticotropic hormone acts on the hypothalamus to decrease corticotropin-releasing hormone secretion, which in turn suppresses its own production and the production of cortisol (21). A healthy stress response is limited in duration, returning the system to baseline levels of cortisol secretion when the stressful event had passed (2,10,18). This ensures that the catabolic, immunosuppressive, and hypervigilant effects of the stress response, among others, are not harmful to the individual (2). However, if this system is chronically activated due to prolonged stress exposure, these control mechanisms may break down, leading to HPA axis dysfunction, known as allostatic load (22). This is commonly observed in studies of individuals who have experienced chronic stress through experiences of maltreatment, trauma, or adverse life events, who show altered cortisol responses compared to healthy controls (7,23). These altered responses can be either abnormally high or low cortisol levels (7). Because the stress response is part of a homeostatic system, it performs best under a baseline level of cortisol secretion (9), and therefore increased allostatic load in chronically stressed individuals and the subsequent over- or under-activation of the cortisol response can result in harmful health outcomes (7,18,22). However, it is currently unclear why individuals exposed to chronic stress or adverse life events have been observed to have over-activation of the cortisol response in some studies and under-activation in others. It has been suggested that the response may be time-dependent, with an initial increase in cortisol output after exposure to the stressor, followed by attenuation and a subsequent decrease in cortisol output, that different stressors may elicit different responses, or that there may be individual differences in the physiological response to chronic stress (7).

1.3 Measurement of stress

Each of the body's responses to stressors, both psychological and physiological, give rise to different approaches to measuring stress. The psychological approach focuses on measuring perceived stress through the individual's perception of the stressors and emotional responses, while the physiological approach measures the body's biological response to the stressors (5). Because perceived stress has typically been easier to assess than physiological stress, it has traditionally been used measure stress in both research and clinical practice (13). However, because perceived stress can be strongly influenced by subjective factors, there has been a growing interest in using objective measures of stress based on physiological biomarkers (5,24).

Perceived stress is typically measured using self-report questionnaires which ask an individual about how stressful they feel their lives, or certain life events, are during a specified period of time (25). They focus on the individual's appraisal and emotional responses to stress, instead of stressors themselves or their physiological response, and highlight the importance of the subjective appraisal of stress (5,25). Questionnaires have been demonstrated to be an appropriate tool for assessing stress in epidemiologic studies and are generally easy and inexpensive to administer (5,25). However, questionnaires assessing perceived stress are limited by the scope of the items included in the questionnaire, the time frame included in the questionnaire, and possible recall or social desirability biases (5,26).

Physiological stress is typically measured by examining the concentration of cortisol in the body (5). Cortisol measurements are preferred to measuring catecholamines because it reflects more chronic stress responses, compared to the transient nature of catecholamines (5). Traditionally this has been done by measuring the cortisol concentration in samples of saliva, urine, or plasma (27). However, these methods only measure acutely circulating cortisol, and can only provide accurate assessments for periods of up to 24 hours and are therefore not able to detect long-term trends in cortisol levels (27). Measuring cortisol concentration from the hair of the scalp is a relatively new method which can measure average cortisol levels over longer periods of time (28). With an average hair growth rate of ~1 cm per month, cumulative cortisol exposure over three months can be measured with a 3 cm hair segment (27). This makes hair cortisol concentration (HCC) a particularly useful measure of long-term physiologic stress and well suited for comparison to perceived stress questionnaires, which often evaluate stress over a similar time frame (5). However, because HCC is a relatively new method for measuring physiological stress, clinically relevant reference ranges have not yet been established for most populations (24,29).

1.4 Psychoendocrine covariance

Psychoendocrine covariance refers to the association between the psychological and physiological components of the stress response (5,8,13). Because these components are thought to act in coordination in response to a stressor, a strong association between their responses is expected (8,13). This hypothesis is supported by the close link between the HPA axis, which regulates the longer-term physiological stress response, and the cortical and limbic structures which control the psychological stress response (8,13). Because of the close relationship between the psychological and physiological components of the stress response, it has been proposed that HCC could be used as a clinical correlate of perceived stress (21). However, despite the theoretical plausibility of this link, associations reported between these measures have been inconsistent (5,13).

In studies specifically examining the association between measures of perceived stress and HCC, results have been unclear. While some studies report a positive association between perceived stress and HCC (21,28,30–33), others have found a negative association (27,34–36), or no association (24,26,37–45). The findings of an inconsistent relationship between perceived stress and HCC may indicate that these measures are not simply two different methods to measure the stress response and should not be used as clinical correlates of one another (24). Instead, they may be capturing two distinct elements of the stress response (24,26,41). This is an important consideration because if each measure is capturing unique aspects of the stress response, both methods may be needed to obtain a comprehensive assessment of stress (26,32).

1.5 Factors that affect the stress response

Several factors have been reported to be associated with both psychological and physiological measures of stress, including age, sex, and the presence of a chronic physical illness, among others. However, some of these factors may have different relationships with psychological and physiological stress, respectively.

For example, older age has typically been shown to be associated with lower levels of perceived stress (33,39,46,47), but HCC has been found to increase with age (48–53). Similarly, while studies typically report higher levels of perceived stress in females than in males (54–58), the opposite association is observed for HCC, with most studies finding higher HCC in males than in females (26,33,51–53,59–66). However, some studies in adolescents have found higher HCC in females (24,67). Individuals with lower income tend to report higher levels of perceived stress (47) and have higher HCC (53,67–69), however one study has found a positive association between caregiver income and HCC in children (50). Higher levels of perceived stress have also been associated with worsening symptoms of chronic physical illnesses such as asthma, inflammatory bowel disease, and epilepsy (70–72). Increased HCC has also been observed in individuals with a wide variety of physical illnesses including heart disease, diabetes, and epilepsy, among others (31,33,48,52,65,73,74). Parent psychopathology has also been associated with increased HCC in children (75), however no studies have yet examined its relationship with perceived stress.

However, despite the apparent relationships between these factors and perceived stress and HCC, many studies have also found no associations between these variables and both perceived stress (24,33,39,76,77) and HCC (24,26,33–35,39,42,43,48–50,53,60,64–66,68,78–94).

1.6 Stress and mental disorder

Mental disorder is defined by the American Psychiatric Association as a disturbance in an individual's cognition, emotion regulation, or behaviour which reflects dysfunction in psychological, biological, or developmental processes (95). It is estimated that one in five Canadians are affected by mental disorders, with similar rates observed in adults and youth (96). Mental disorders are commonly classified into internalizing and externalizing disorders. Those with internalizing disorders tend to express distress inwards, while those with externalizing disorders tend to express distress outwards (97). Common internalizing disorders include mood disorders (e.g., major depressive disorder) and anxiety disorders (e.g., generalized anxiety disorder, separation anxiety, and phobias) (97). Common externalizing disorders include attention-deficit hyperactivity disorder, oppositional defiant disorder, and conduct disorder (97).

Exposure to chronic stress has been linked to vulnerability to poorer health outcomes, including mental disorders (7,19,98). However, the association between measures of psychological and physiological stress and mental disorder is unclear. While mental disorders are typically associated with higher levels of perceived stress (34,37,39,42,46,54,55,76,98–103), the association between mental disorder and HCC is not as clear. Several studies have reported a similar positive association between HCC and mental disorders (28,63,67,75,84,104–106). However, others have reported negative associations (34,37,67,75,107,108), or no association (39,42,45,51,60,84,90,106).

It has also been suggested that the association between HCC and mental disorder may be disorder-dependent with some disorders being associated with increased HCC, while other are associated with decreased HCC (19,51,67,75,84). Anxiety disorders and externalizing disorders, such as attention-deficit hyperactivity disorder and oppositional defiant disorder, are typically associated with lower HCC (51,63,67,75,107,108). However, depression has been found to be associated with both higher (28,67,104,105) and lower HCC (34,37,75). A recent study has also found evidence that HCC may have a curvilinear association with depression, with both high and low levels of cortisol being associated with depressive symptoms, which may explain some of the inconsistent findings in previous studies (109). This aligns with findings that individuals who have experienced chronic stress show both over- and under-activation of the cortisol response (7). However, these studies have been conducted using a wide variety of populations and the true associations between HCC and various mental disorders remains unclear.

STUDY RATIONALE AND RESEARCH OBJECTIVES

Stress is a common topic of research, particularly in its relationship to mental health (7,19). However, there is no standard method for measuring stress, resulting in a number of different approaches being used, including measures of both the psychological and physiological components of the stress response (5). While these responses are thought to be interconnected, studies examining their association have yielded inconsistent results (5,13). Therefore, elucidating the relationship between perceived stress questionnaires and HCC, the two most common methods for assessing chronic stress, is important to ensure the comprehensive evaluation of stress in both future research and clinical practice.

Despite this, research examining the relationship between perceived stress and HCC is limited, and no studies have previously assessed the agreement between these two measures. It is important to assess the agreement between these measures, and not just their correlation or association with one another because these methods only assess the linear association between the two measures. Estimating the agreement goes beyond assessing if the measures are related and allows us to assess systematic differences between the measures (110). Further, while many studies have studied individual risk factors for HCC or perceived stress, and their associations with mental disorders, none have directly compared these associations in the same sample.

Understanding if measures of psychological and physiological stress agree with each other and evaluating their associations with various risk factors and mental disorders is important to determine if they can be used as independent measures of stress, or if they are each capturing different elements of the stress response. This is particularly relevant in a clinical population because of the suggested association between stress and mental disorder, where the selection of appropriate and comprehensive methods for stress assessment is therefore especially important.

The current study explored the relationship between measures of perceived stress and HCC in a clinical sample of youth with mental disorder and their parents. The specific objectives of this study were to:

1. Estimate the agreement between measures of perceived stress and HCC.

It was hypothesized that there would be low to moderate agreement between perceived stress and HCC for both the youth and parent subgroups.

2. Model and compare the sociodemographic and psychosocial risk factors for elevated levels of perceived stress and HCC.

It was hypothesized that sociodemographic and psychosocial variables would be associated with both perceived stress and HCC in both the youth and parent subgroups. It was also hypothesized that the directionality or magnitude of these associations may be different for perceived stress and HCC for some variables such as age and sex.

3. Model and compare the magnitude of the association between mental disorder and perceived stress and HCC, respectively.

It was hypothesized that perceived stress would be positively associated with all mental disorders, while HCC would be negatively associated with generalized anxiety disorder, separation anxiety, phobia, attention-deficit hyperactivity disorder, and oppositional defiant/conduct disorder, and may be either positively or negatively associated with depression.

METHODS

3.1 Study design and sample

The data for this thesis come from a cross-sectional study designed to investigate multimorbidity and mental health service use among youth with mental disorder (111). This study received relevant ethical approvals. Youth were eligible for the study if they were aged 4-17 years; were classified as having major depressive disorder, generalized anxiety disorder, separation anxiety, social phobia, specific phobia, attention-deficit hyperactivity disorder, oppositional defiant disorder, or conduct disorder; were currently receiving inpatient or outpatient mental health services; and had parents with sufficient English skills to complete the in-person psychiatric screening interview and psychosocial and health service use questionnaires. Youth were excluded if their current mental state limited their ability to complete the interview and questionnaires. The parents included in the study were the primary caregiver of the youth. A total of 259 eligible youth was identified. Of these, 144 (56%) provided informed consent, and 100 (39%) participated in the study. Eight youth did not complete the questionnaires, resulting in a final sample of 92 (36%) youth-parent dyads. Youth participating in the study ranged from 8-17 years old.

3.2 Data collection

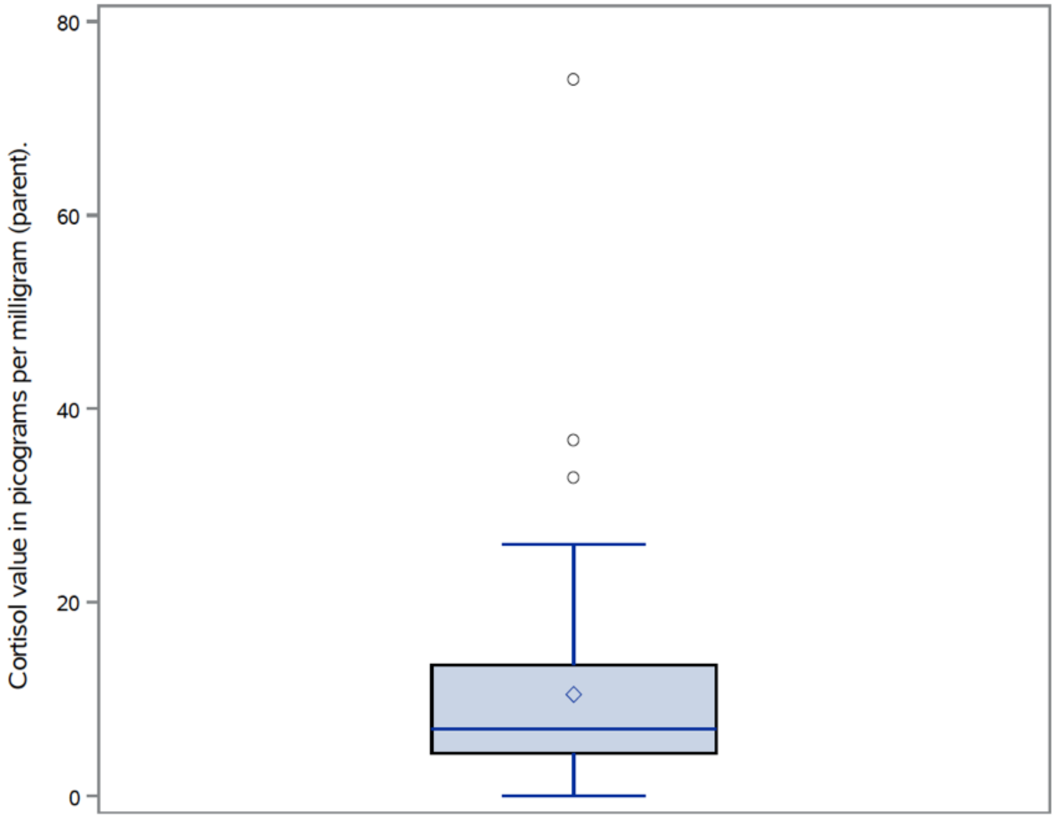
Research staff identified eligible inpatient youth in consultation with the charge nurse and approached potential participants during treatment breaks, introducing the study and explaining what participation would entail. If they were interested, youth gave research staff permission to contact their parents and obtain oral consent for their child to participate in the study. Research staff then scheduled a time for parents to complete the interview, questionnaires, and provide hair samples, which occurred during hospital visits or when the youth was discharged. After receiving oral consent from parents, research staff obtained informed written consent or assent and completed the interview, questionnaires, and obtained the hair sample from the youth.

Youth outpatient rosters, containing contact information of age-eligible youth and parents who agreed to be contacted about research studies, were also provided to research staff for the purpose of recruitment. Research staff contacted families by phone to introduce the study and schedule a time for data collection. All data collection and consent for outpatients occurred at the research office. Interview and questionnaire responses were obtained electronically from parents and youth. Parents provided written consent for themselves and their children < 16 years of age. Children 8-15 years provided written assent and children ≥ 16 years provided written consent.

3.3 Analytical sample

The analytical sample for this thesis included 48 youth aged 14-17 and 73 parents who provided a hair sample and completed the Perceived Stress Scale (PeSS) or Parental Stress Scale (PaSS), respectively. One parent was determined to have an outlying HCC value (Figure 1), and was excluded from the analyses, leaving a final analysis population of 48 youth and 72 parents.

Figure 1: Distribution of parent hair cortisol concentration (pg/mg)



3.4 Study measures and variables

3.4.1 Youth perceived stress

Perceived stress was measured in youth 14-17 using the Perceived Stress Scale (Appendix A). The PeSS is a 10-item self-report scale measuring the perception of stress during the last month (76). The items are designed to assess how unpredictable, uncontrollable, and overloaded individuals feel (76). Responses were scored from '0' (never) to '4' (very often) for a total score between 0 and 40, with higher scores indicating greater appraisal of life situations as stressful (76). The PeSS has been psychometrically validated in a variety of populations (112) and the internal consistency in this sample was $\alpha = 0.87$.

3.4.2 Parent perceived stress

Parent perceived stress was measured using the Parental Stress Scale (Appendix B). The PaSS is an 18-item self-report scale designed to measure the perception of parental stress of both mothers and fathers (113). The items on the PaSS ask parents about their typical relationship with their child and aim to capture both the demands and rewards of parenting (114). Responses were scored from '1' (strongly disagree) to '5' (strongly agree) for a total score between 18 and 90, with higher scores indicating higher levels of parental stress. The PaSS has shown strong psychometric properties in populations of parents of children with and without clinical problems (77,113,115). In this sample, the internal consistency of the PaSS was $\alpha = 0.83$.

3.4.3 Hair cortisol concentration

Hair cortisol concentration was used to measure physiological stress in both youth and their parents. Research staff collected hair samples from parents and youth during the data collection interview. For participants with longer hair, approximately 50-60 hairs were cut from the posterior vertex, as close to the scalp as possible. If a participant's hair was shorter, 15-20 hairs were collected from four-to-five different locations. Samples were clipped to cardstock indicating the direction of hair growth. Parents completed two standardized questionnaires, one for themselves and one for their child, that included variables hypothesized to affect HCC (medication use, hair length and color, hair washing and treatments, smoke exposure, ethnicity) (75,84).

Hair samples were analyzed using a standardized protocol for washing, extraction, and cortisol assays (68). The first 3 cm of each hair sample proximal to the scalp was cut and placed into a 50 mL Falcon conical centrifuge tube. Each sample was washed twice with 12 mL of isopropanol and shaken by hand for 2 minutes. Tubes were left open for 48 hours to air dry. Dried samples were placed in a grinding jar with four stainless steel ball bearings and ground using the Retsch CryoMill at 25 Hz for three minutes. 30-35 mg hair ground hair powder was placed in a 2 mL Eppendorf tube with 1 mL of 1.0% ethanol, shaken by hand, and rotated at 45 rpm on the RPI Mix-All Laboratory Tube Mixer for 24 to 72 hours at room temperature. During the first extraction, samples were vortexed for 5 seconds and centrifuged at 2800 rpm for 15 minutes and 0.8mL of supernatant was aliquoted into a new 2 mL Eppendorf tube. The supernatant was left to air dry for 48 hours to allow the ethanol to evaporate completely. Next, another 1 mL or 1.0% ethanol was added to the original sample tube and it was rotated at 45 rpm on the RPI Mix-All Laboratory Tube Mixer for 24 to 72 hours at room temperature. For the second extraction, the same steps were followed, except 1.0 mL of supernatant was aliquoted. The supernatant was reconstituted with 150 μL of Salimetrics Salivary Cortisol Assay Diluent, vortexed for 5 seconds, and centrifuged for 10 minutes. The samples were then assayed in duplicate by high-sensitivity enzyme-linked immunosorbent assays using the High Sensitivity Salivary Cortisol Immunoassay Kit (Cat# 1-3002, Salimetrics, Pennsylvania), as per manufacturer instructions. The protocol for the current study opted to use 1.0% ethanol as opposed to methanol as described by Vaghri et al. (2013; Ferro & Gonzalez, 2020). A pilot test was run and determined that the values were highly correlated and therefore laboratory technicians opted for the less toxic and abrasive ethanol. Cortisol levels are expressed as pg/mg of hair. Intra and inter- assay coefficients of variance were <10 % (75).

3.4.4 Youth mental disorder

The Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID) was used to assess the most common internalizing disorders (major depressive disorder, separation anxiety disorder, social phobia, specific phobia, and generalized anxiety disorder) and externalizing disorders (attention-deficit hyperactivity disorder, oppositional defiant disorder, and conduct disorder) (116,117). The MINI-KID is a structured diagnostic interview that assesses DSM-IV and ICD-10 disorders in youth up to 17 years (118). It is composed of diagnostic modules containing screening questions for each disorder. If screening questions are endorsed, the respondent completes the questions within the module to determine if the child meets the disorder criteria, otherwise the module is skipped (118). Responses to the MINI-KID are binary (yes/no for each disorder). The MINI-KID was administered to youth, answering questions about themselves, and parents, answering questions about their child. The MINI-KID has demonstrated strong psychometric properties (119,120) and has been validated against the Schedule for Affective Disorders and Schizophrenia for School Aged Children-Present and Lifetime Version (118).

Due to the limited sample size available for these analyses and low prevalence of some disorders studied, social phobia and specific phobia were combined into a single category (now referred to as 'phobia') and oppositional defiant disorder and conduct disorder were combined into a single category ('now referred to as 'oppositional defiant/conduct disorder').

3.4.5 Parent psychopathology

Symptoms of depression and anxiety were measured using The Center for Epidemiological Studies Depression Scale (CES-D) and The State Trait Anxiety Inventory (STAI).

The CES-D is a 20-item self-report scale designed to assess depressive symptoms over the past week in adults (121). The CES-D includes items that assess depressive affect, positive affect, somatic activity, and interpersonal relationships (121). It uses a four-point Likert scale (0-3) with anchor points for frequency of symptoms ranging from ranging from "rarely or none of the time (< 1 day)" to "most or all of the time (5–7 days)." Total scores range from 0 to 60, with higher scores indicating greater impairment and scores ≥ 16 typically indicating clinically relevant levels of depressive symptoms (121). The CES-D has been psychometrically validated (77,122,123) and the internal consistency in this sample was $\alpha = 0.86$.

The STAI is a 40-item measure for measuring anxiety in adults (124). For this study, only the 20 items measuring "trait anxiety" were used which assess how individuals "generally feel". Responses were scored from '1' (almost never) to '4' (almost always) for a total score between 20 and 80, with higher scores indicating higher levels of anxiety. The STAI has been psychometrically validated (77,123–125) and the internal consistency in this sample was $\alpha = 0.75$.

Due to the limited sample size available for this study, the limited number of covariates that could be included, and the high correlation between the two scales ($r = 0.72$), for analyses in the youth subgroup, parent's CES-D and STAI scores were added together to create a composite measure of 'parent psychopathology'.

3.4.6 Family functioning

Family functioning was measured using the General Functioning subscale of the McMaster Family Assessment Device (FAD), completed by parents. The FAD is a 12-item self-report measure of the overall health/pathology of the family (126). The items represent problem solving, communication, roles, affective responsiveness, affective involvement, and behaviour control (127). Responses were scored on a 4-point scale from

‘0’ (strongly agree) to ‘3’ (strongly disagree) and summed for a total score out of 36, with higher scores representing better family functioning (127). The FAD has been psychometrically validated (128) and the construct validity of the General Functioning subscale has been established (127,129). In this sample, the internal consistency of the FAD was $\alpha = 0.81$.

3.4.7 Chronic physical illness

Presence of a physical illness in youth was measured using a question adapted from the Canadian Community Health Survey-Mental Health. Parents were asked if their child had been diagnosed with any chronic physical conditions. If they answered yes, they were asked to specify the physical condition that their child had been diagnosed with.

3.4.8 Sociodemographic characteristics

Relevant sociodemographic variables were also collected. These included youth and parent age and sex, and household income. Household income was coded as annual household income above or below $90,000, the Canadian median household income for ≥2 person households (130).

3.5 Data analysis

All statistical analyses were conducted with SAS Studio, Version 9.0.4. Due the exploratory nature of this study, all analyses were conducted using a significance level of $\alpha = 0.10$ (131).

3.5.1 Objective 1

Bland-Altman plots were constructed to assess the agreement between HCC and perceived stress scores for both the youth and parent subgroups. Because Bland-Altman plots are designed to compare two measures which use the same scale both HCC and PeSS/PaSS scores were transformed to z-scores before being plotted so they could be directly compared. The plots were constructed by plotting the difference between HCC and PeSS or PaSS score on the Y-axis and the mean of the two measures on the X-axis. The line of identity, representing perfect agreement between HCC and perceived stress score, was plotted at $Y = 0$ on both plots. The limits of agreement were also plotted at 1.65 standard deviations from the mean, representing 90% confidence limits, on both plots.

The intraclass correlation coefficient (ICC) was used as a quantitative measure of agreement. The ICC (and corresponding 90% confidence intervals) was calculated between HCC and perceived stress scores for both the youth and parent subgroups. ICC < 0.50 indicated poor reliability, ICC = 0.50 – 0.75 moderate reliability, ICC = 0.75 – 0.90 good reliability, and ICC > 0.90 excellent reliability (132).

3.5.2 Objective 2

Multiple linear regression was used to model the association between selected covariates and HCC and perceived stress scores for both youth and parents (using the PeSS scores for youth and PaSS scores for parents).

Two groups of models were generated for each subgroup with the covariates of interest as the predictor variables and HCC or perceived stress as the continuous outcome variables. Within each group, each model sequentially added blocks of covariates to assess their incremental effects within the models. The full models were used to examine the association between the covariates of interest and HCC/perceived stress.

Hair cortisol concentration, one of the outcome variables of interest, was not normally distributed in either the youth or parent subgroups. Thus, log-transformed HCC values, which more closely approximate a normal distribution, were used as the outcome variable for these analyses (Figures 2 & 3).

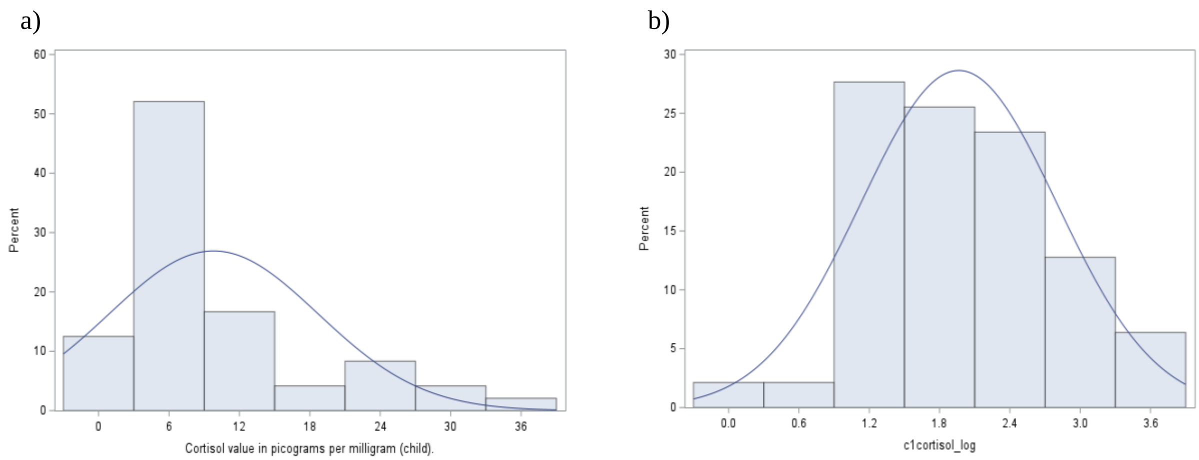


Figure 2: Distribution of hair cortisol concentration in youth (a) before and (b) after log transformation

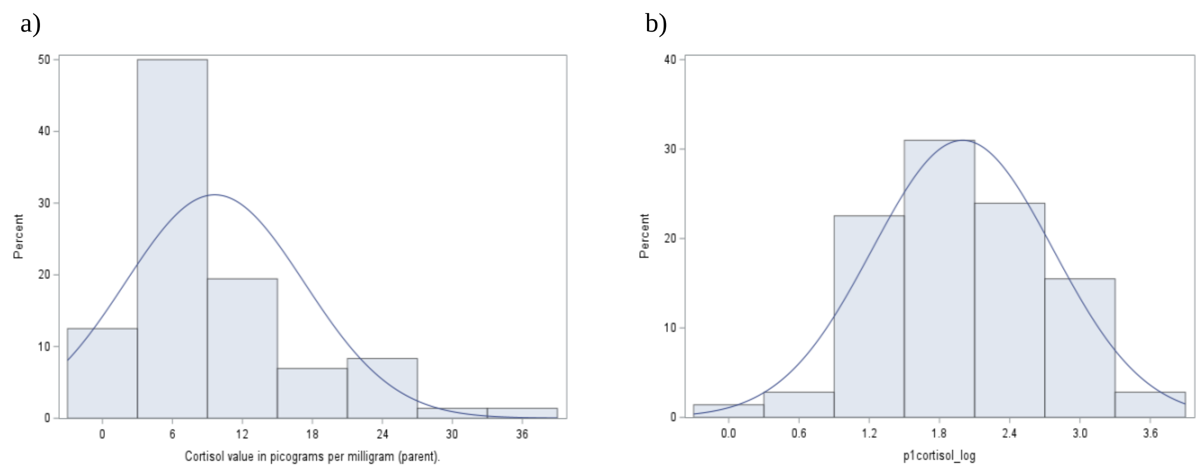


Figure 3: Distribution of hair cortisol concentration in parents (a) before and (b) after log transformation

PROC REG was used to generate two models using PeSS score as the outcome variable and two models using log-transformed HCC as the outcome variable for the youth subgroup (Appendix C). The first model in each set included sociodemographic characteristic – youth age, sex, and household income. The second model added psychosocial and clinical characteristics – presence of a chronic physical illness and parent psychopathology (using combined CES-D and STAI scores).

PROC REG was also used to generate three models using PaSS score as the outcome variable and three models using log-transformed HCC as the outcome variable for the parent subgroup (Appendix C). The first model in each set included sociodemographic characteristics for the parent – age, sex, and household income. The second model added demographic and clinical characteristics for their child – youth age, sex, and presence of a chronic physical illness. The third model added family functioning.

To compare the relative effect of each covariate on perceived stress scores and HCC, the method of variance estimates recovery (MOVER) was used to calculate the differences in estimates and associated 90% confidence intervals (133). The MOVER is an approach which constructs confidence intervals around a difference between two measures of association (133). Using the MOVER, it can be determined if the differences between two measures of association are statistically significant. For this study, the MOVER was used to compare the associations for each risk factor and perceived stress and HCC, respectively, to determine if each risk factor was more strongly associated with one measure of stress than the other.

3.5.3 Objective 3

Binary logistic regression was used to model the association between perceived stress or HCC and mental disorder in youth. The PROC LOGISTIC procedure was used to create models with PeSS score or HCC as the predictor variable and mental disorder as indicated by the youth on the MINI-KID as the binary outcome variable. Two models were created for each mental disorder (major depressive disorder, generalized anxiety disorder, attention-deficit hyperactivity disorder, oppositional defiant/conduct disorder, phobia, and separation anxiety), one with PeSS score as the predictor variable and one with HCC as the predictor variable, for a total of 12 models (Appendix D). Youth age and sex were also included in all models as control variables.

Multiple linear regression was used to model the association between perceived stress or HCC and mental disorder in parents. The PROC REG procedure was used to create models with PaSS score or HCC as the predictor variable and either depression or anxiety as indicated by the parent on the CES-D or STAI, respectively, as the continuous outcome variable. Two models were created for each disorder, one with PaSS score as the predictor variable and one with HCC as the predictor variable, for a total of four models (Appendix D). Parent age and sex were also included in all models as control variables.

RESULTS

4.1 Sample characteristics

4.1.1 Youth subgroup

Sample characteristics for the youth subgroup are presented in Table 1. Youth had a mean age of 15.6 (SD 1.2) years, 81% were female, and 26% had a chronic physical illness. Sixty-three percent of youth had a household income < $90,000. Youth had a mean HCC of 9.8 pg/mg (SD 8.9) and PeSS score of 26.4 (SD 8.0). The most commonly reported mental disorders among youth were major depressive disorder (79%), phobia (75%), and generalized anxiety disorder (67%).

Table 1: Sample characteristics for the youth subgroup (n=48)

Variable	**N (%) or Mean (SD)**
Age (years)	15.63 (1.16)
Female	38 (80.85%)
Household income < $90,000	30 (62.50%)
Presence of a chronic physical illness	12 (25.53%)
Parent's combined CES-D + STAI score	65.91 (16.81)
HCC (pg/mg)	9.80 (8.90)
PeSS score	26.40 (7.97)
Mental disorder	
Major depressive disorder	38 (79.17%)
Generalized anxiety disorder	32 (66.67%)
Separation anxiety	13 (27.08%)
Phobia	36 (75.00%)
Attention-deficit hyperactivity disorder	18 (37.50%)
Oppositional defiant/conduct disorder	20 (41.67%)

SD = Standard Deviation.

4.1.2 Parent subgroup

Sample characteristics for the parent subgroup are presented in Table 2. Parents had a mean age of 45.5 (SD 6.7) years, and 90% were female. Sixty-one percent of parents had a household income < $90,000. Parents had a mean HCC of 9.6 pg/mg (SD 7.7) and PaSS score of 51.7 (SD 12.7). Two-thirds of their children were female, they had a mean age of 13.6 (SD 3.2), and 26% had a chronic physical illness. Parents had a mean CES-D score of 20.3 (SD 10.1) and STAI score of 43.9 (SD 7.4).

Table 2: Sample characteristics for the parent subgroup (n=72)

Variable	**N (%) or Mean (SD)**

Age (years)	45.49 (6.74)
Female	65 (90.28%)
Household income < $90,000	44 (61.11%)
Child's age (years)	13.58 (3.24)
Female child	48 (66.67%)
Child with chronic physical illness	19 (26.39%)
FAD score	20.22 (5.95)
HCC (pg/mg)	9.63 (7.68)
PaSS score	51.65 (12.72)
CES-D score	20.27 (10.11)
STAI score	43.92 (7.38)

SD = Standard Deviation.

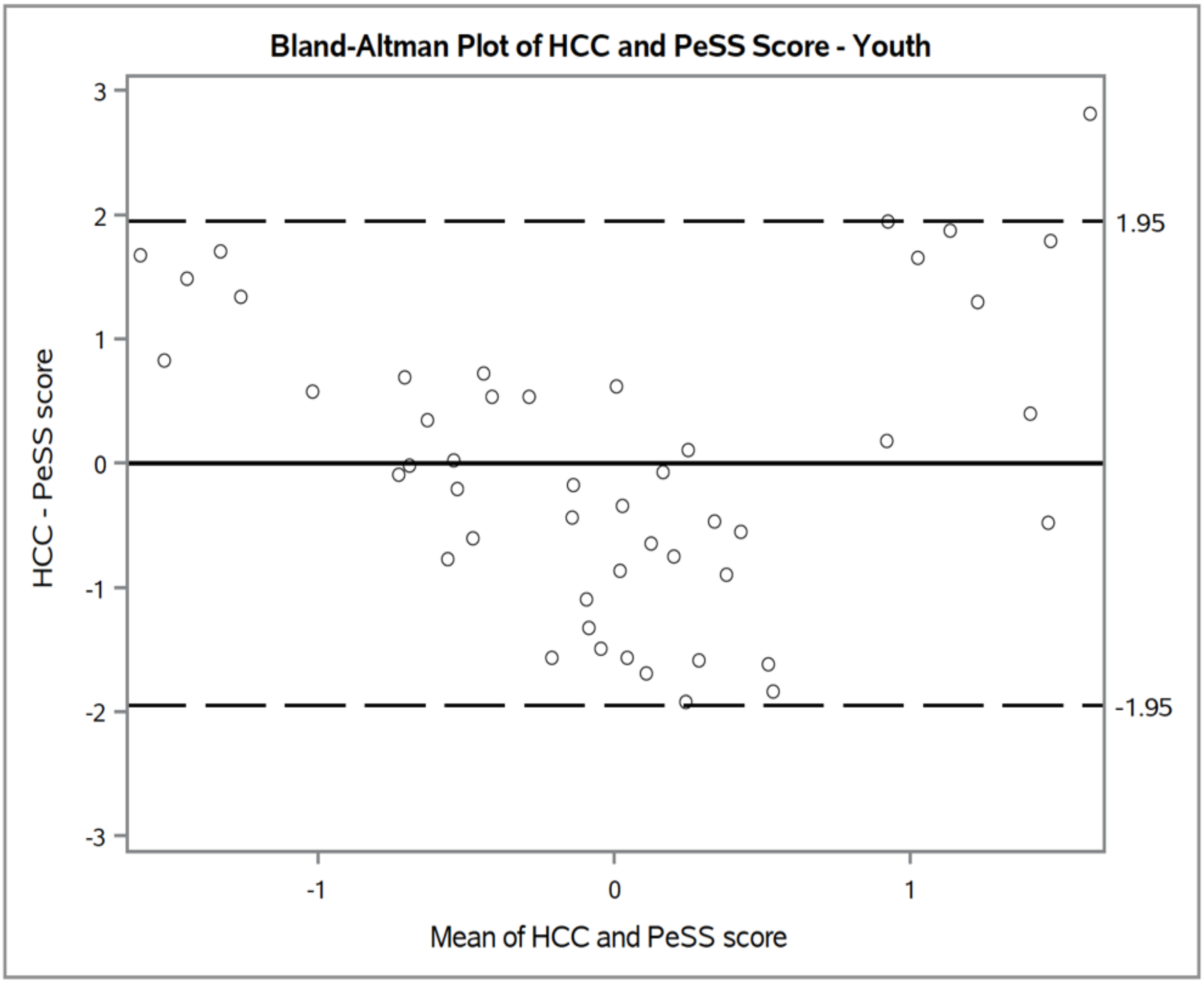
4.2 Objective 1

4.2.1 Youth subgroup

The Bland-Altman plot showing agreement between HCC and PeSS score in the youth subgroup is shown in Figure 4. In the youth subgroup, the points are not equally and randomly distributed around the line of identity, indicating that there are systematic differences between the measures. Specifically, when stress levels were high or low (as indicated by the mean z-score of HCC and PeSS score), most of the points lie above the line of identity, indicating that HCC overestimated stress levels, relative to PeSS score. However, when stress levels were moderate, most of the points lie below the line of identity, indicating PeSS score overestimated stress levels, relative to HCC.

The 90% limits of agreement were calculated to be ±1.95 for the youth subgroup (Figure 4). The ICC between HCC and PeSS scores (0.31 [90% CI 0.08, 0.51]) indicated poor reliability between the measures.

Figure 4: Bland-Altman plot for HCC and PeSS scores in the youth subgroup



4.2.2 Parent subgroup

The Bland-Altman plot showing agreement between HCC and PaSS score in the parent subgroup is shown in Figure 5. As in the youth subgroup, in the parent subgroup the points are not equally and randomly distributed around the line of identity, indicating that there are systematic differences between the measures. Specifically, when stress levels were low (as indicated by the mean z-score of HCC and PaSS score), most of the points lie above the line of identity, indicating HCC overestimated stress levels, relative to PaSS score. At moderate and high levels of stress, there was a greater variability in the differences between the two measures, with an equal distribution of points above and below the line of identity. The 90% limits of agreement were calculated to be ±2.17 for the parent subgroup (Figure 5). As in the youth subgroup, the ICC between HCC and PeSS scores (0.15 [90% CI -0.04, 0.33]) indicated poor reliability.

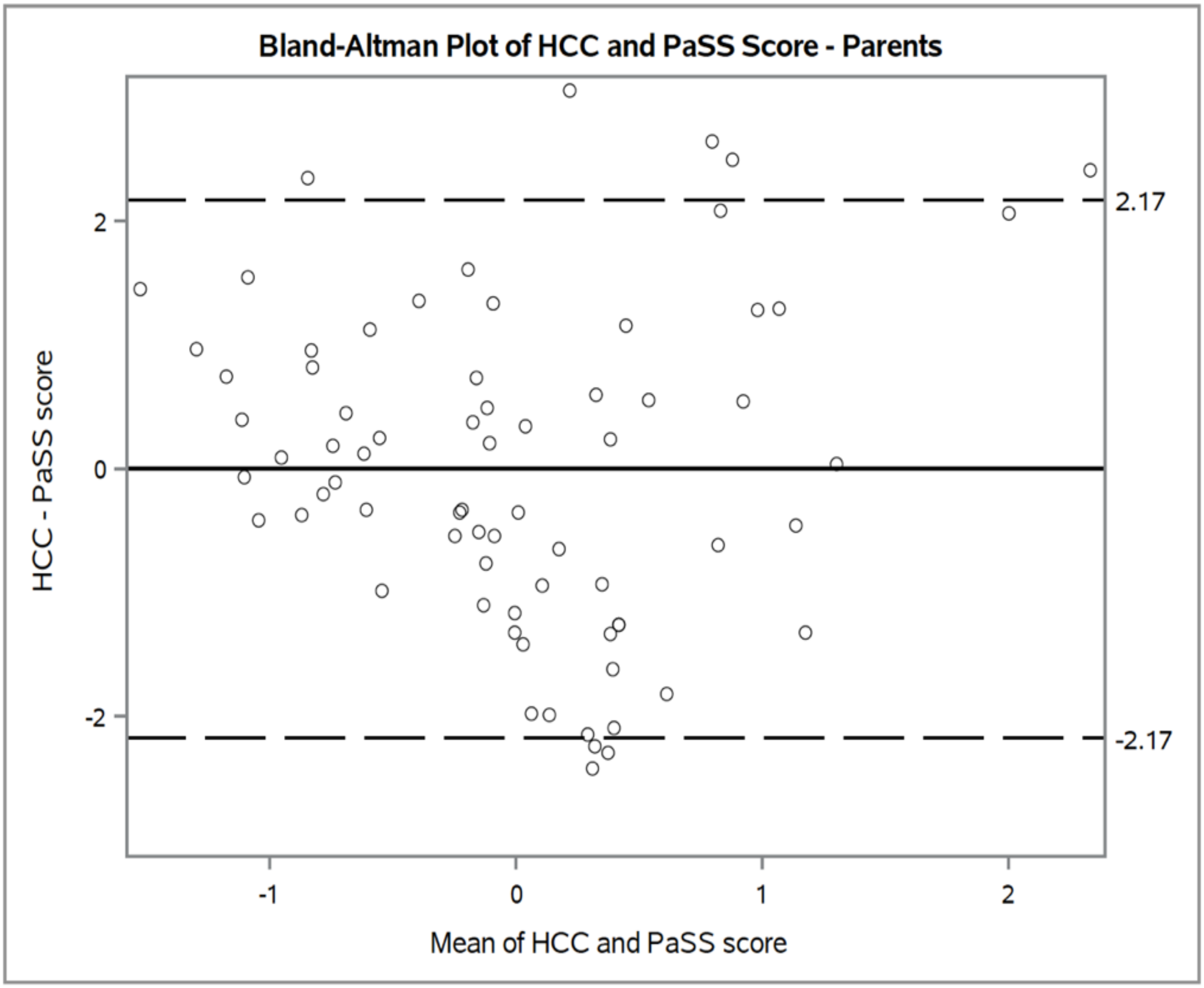


Figure 5: Bland-Altman plot for HCC and PaSS score in the parent subgroup

4.3 Objective 2

4.3.1 Youth subgroup

Results of the linear regression analyses in the youth subgroup, for both HCC and PeSS scores, are displayed in Table 3. In the first set of models, examining sociodemographic characteristics, having a household income < $90,000 was associated with higher HCC (*β* = 0.33 [90% CI 0.80, 0.57]) and female sex was associated with higher PeSS scores (*β* = 0.36 [90% CI 0.12, 0.60]). When adding parent psychopathology and the presence of a chronic physical illness into the models, both associations remained significant (*β* = 0.24 [90% CI 0.00, 0.47] and *β* = 0.42 [90% CI 0.19, 0.65], respectively). Additionally, higher scores on measures of parent psychopathology were associated with increased HCC and PeSS scores (*β* = 0.42 [90% CI 0.19, 0.64] and *β* = 0.28 [90% CI 0.06, 0.51], respectively), while the presence of a chronic physical illness was associated with lower PeSS scores (*β* = -0.24 [90% CI -0.46, -0.01]).

Table 3: Linear regression of covariates of interest on HCC/PeSS scores in the youth subgroup

	Model 1	**Model 2**
HCC		
Adjusted R^2	0.09	0.23
Age	0.21 (-0.05, 0.47)	0.20 (-0.04, 0.44)
Female sex	-0.22 (-0.48, 0.04)	-0.18 (-0.42, 0.06)
Household income < $90,000	0.33 (0.80, 0.57) *	0.24 (0.00, 0.47) *
Parent's combined CES-D + STAI score		0.42 (0.19, 0.64) *
Presence of a chronic physical illness		0.04 (-0.19, 0.26)
PeSS		
Adjusted R^2	0.14	0.23
Age	0.11 (-0.13, 0.35)	0.09 (-0.13, 0.32)
Female sex	0.36 (0.12, 0.60) *	0.42 (0.19, 0.65) *
Household income < $90,000	0.17 (-0.06, 0.41)	0.10 (-0.33, 0.13)
Parent's combined CES-D + STAI score		0.28 (0.06, 0.51) *
Presence of a chronic physical illness		-0.24 (-0.46, -0.01) *

Values denote standardized *B*-coefficient (90% CI). * Denotes statistical significance at p<0.10.

When comparing the strength of the associations between the covariates and HCC/PeSS score in the final models, a noteworthy difference was observed for youth sex ($\Delta\beta$ = -0.60 [-0.93, -0.27]), which was more strongly associated with PeSS score than HCC (Table 4).

Table 4: Results of the MOVER analysis for the youth subgroup

	$\Delta\beta$ (90% CI)
Age	0.11 (-0.22, 0.44)
Female sex	-0.60 (-0.93, -0.27) *
Household income < $90,000	0.14 (-0.10, 0.63)
Parent's combined CES-D + STAI score	0.14 (-0.19, 0.45)
Presence of a chronic physical illness	0.28 (-0.05, 0.59)

* Denotes statistical significance. CI = Confidence Interval.

4.3.2 Parent subgroup

Results of the linear regression analyses in the parent subgroup, for both HCC and PaSS scores, are displayed in Table 5. In the first set of models, examining sociodemographic characteristics of the parent, female sex was associated with lower HCC (β = -0.21 [90% CI -0.41, -0.02]) and having a household income <$90,000 was associated with higher HCC (β = 0.19 [90% CI 0.00, 0.39]). No covariates were significantly associated with PaSS score. When adding demographic and clinical characteristics for their child, only female sex remained significantly associated with HCC (β = -0.21 [90% CI -0.41, -0.00]). In the final models, which added FAD score (as a measure of family functioning), female sex remained associated with lower HCC (β = -0.21 [90% CI -0.42, -0.00]), and FAD score was associated with higher PaSS scores (β = 0.46 [90% CI 0.26, 0.65]).

Table 5: Linear regression of covariates of interest on HCC/PaSS scores in the parent subgroup

	Model 1	Model 2	Model 3
HCC			
Adjusted R^2	0.08	0.05	0.04
Age	0.16 (-0.04, 0.36)	0.15 (-0.07, 0.37)	0.16 (-0.07, 0.39)
Female sex	-0.21 (-0.41, -0.02) *	-0.21 (-0.41, -0.00) *	-0.21 (-0.42, -0.00) *
Household income < $90,000	0.19 (0.00, 0.39) *	0.19 (-0.01, 0.39)	0.19 (-0.01, 0.39)
Child's age		0.02 (-0.21, 0.24)	0.02 (-0.21, 0.25)
Female child		0.07 (-0.13, 0.28)	0.08 (-0.13, 0.29)
Child with chronic physical illness		-0.09 (-0.29, 0.12)	-0.08 (-0.29, 0.12)
FAD score			0.03 (-0.18, 0.24)
PaSS			
Adjusted R^2	-0.03	-0.06	0.14
Age	-0.11 (-0.32, 0.09)	-0.08 (-0.31, 0.15)	0.05 (-0.17, 0.26)
Female sex	-0.05 (-0.26, 0.15)	-0.06 (-0.27, 0.15)	-0.09 (-0.28, 0.10)
Household income < $90,000	-0.03 (-0.23, 0.17)	-0.02 (-0.23, 0.18)	-0.03 (-0.21, 0.16)
Child's age		-0.07 (-0.30, 0.16)	-0.04 (-0.25, 0.17)
Female child		-0.09 (-0.31, 0.12)	-0.03 (-0.23, 0.17)
Child with chronic physical illness		0.07 (-0.14, 0.27)	0.09 (-0.10, 0.27)
FAD score			0.46 (0.26, 0.65) *

Values denote standardized *B*-coefficient (90% CI). * Denotes statistical significance at $p<0.10$.

When comparing the strength of the association between the covariates and HCC/PaSS score in the final models, a noteworthy difference was observed for family functioning ($\Delta\beta$ = -0.43 [-0.71, -0.14]), which was more strongly associated with PaSS score than HCC (Table 6).

Table 6: Results of the MOVER analysis for the parent subgroup

	$\Delta\beta$ (90% CI)
Age	0.11 (-0.20, 0.43)
Female sex	-0.12 (-0.40, 0.16)
Household income < $90,000	0.22 (-0.06, 0.49)
Child's age	0.06 (-0.25, 0.37)
Female child	0.11 (-0.18, 0.40)
Child with chronic physical illness	-0.17 (-0.38, 0.11)
FAD score	-0.43 (-0.71, -0.14) *

* Denotes statistical significance. CI = Confidence Interval.

4.4 Objective 3

4.4.1 Youth subgroup

The results of the logistic regression analyses between HCC/PeSS score and mental disorder in youth are shown in Table 7. When controlling for age and sex, higher HCC was associated with increased odds of generalized anxiety disorder (OR = 1.14 [90% CI 1.01, 1.28]) and higher PeSS scores were associated with increased odds of major depressive disorder (OR = 1.33 [90% CI 1.12, 1.57]), generalized anxiety disorder (OR = 1.10 [90% CI 1.01, 1.19]), and separation anxiety (OR = 1.14 [90% CI 1.03, 1.25]).

Table 7: Logistic regression of HCC/PeSS scores on mental disorder in the youth subgroup

	HCC	**PeSS**
Major depressive disorder	1.05 (0.95, 1.16)	1.33 (1.12, 1.57) *
Generalized anxiety disorder	1.14 (1.01, 1.28) *	1.10 (1.01, 1.19) *
Separation anxiety	1.04 (0.98, 1.10)	1.14 (1.03, 1.25) *
Phobia	1.01 (0.94, 1.08)	1.06 (0.98, 1.14)

Attention deficit hyperactivity disorder	0.98 (0.92, 1.04)	1.03 (0.96, 1.11)
Oppositional defiant/conduct disorder	0.98 (0.93, 1.04)	0.99 (0.93, 1.06)

Values denote Odds Ratio (90% CI). * Denotes statistical significance at p<0.10.

4.2.2 Parent subgroup

The results of the linear regression analyses between HCC/PaSS and CES-D and STAI score are shown in Table 8. When controlling for age and sex, higher HCC was significantly associated with increased CES-D scores (β = 0.27 [90% CI 0.06, 0.48]) and higher PaSS scores were significantly associated with increased CES-D and STAI scores (β = 0.53 [90% CI 0.35, 0.71] and β = 0.45 [90% CI 0.26, 0.64], respectively). HCC was not significantly associated with STAI score.

Table 8: Linear regression of HCC/PeSS scores on CES-D and STAI score in the parent subgroup

	HCC	**PaSS**
CES-D	0.27 (0.06, 0.48) *	0.53 (0.35, 0.71) *
STAI	0.15 (-0.07, 0.37)	0.45 (0.26, 0.64) *

Values denote standardized *B*-coefficient (90% CI). * Denotes statistical significance at p<0.10.

DISCUSSION

5.1 Objective 1 – Psychological and physiological stress agreement

The hypothesis that agreement between perceived stress and HCC would be low to moderate was supported, as the Bland-Altman plots and ICC values suggest that agreement was low in both the youth and parent subgroups. These findings align with other studies examining the association between measures of perceived stress and HCC, which found weak or no associations between perceived stress and HCC in a variety of populations (21,24,26–28,30–35,37–45,51). This provides support to the notion that despite the theoretical link between the psychological and physiological stress responses, there is a lack of psychoendocrine covariance between measures of perceived stress and HCC.

The low agreement found between perceived stress and HCC could be because these measures are capturing two distinct elements of the stress response. The findings in these samples align with the lack of psychoendocrine covariance seen in other studies (21,24,26–28,30–35,37–45,51). This points to the possibility that, despite the theoretical link between perceived stress and HCC as markers of the psychological and physiological components of the stress response, they may be qualitatively different dimensions of the stress response (26). Future studies should further explore the relationship between measures of psychological and physiological stress to determine if the lack of observed agreement is due to factors such as differing timepoints captured by measures of perceived stress or HPA axis dysfunction in individuals who have experiences trauma or adversity, or if it indicates a true lack of psychoendocrine covariance.

There are also several factors which may account for these findings. First, the different time periods captured between the perceived stress questionnaires used and the hair sample that was taken could have contributed to some of the differences between the measurements. Both the PeSS and PaSS ask respondents about their stress during the past month (76,113), while the 3 cm hair segment used to measure HCC is thought to reflect cortisol levels over the past three months (27). Though the time periods captured between the measures are similar, and much more aligned than previous studies using acute cortisol measurements, it is possible that the difference in time frame could account for some of the discrepancies observed between the measures (26,40,41). While perceived stress is typically chronic over time and some studies have reported maintained perceived stress levels over periods of up to two years (134,135), others have found changes in perceived stress scores in as little as six weeks (112,136). Future studies are needed to determine if perceived stress is sufficiently maintained over time for one-month measurements to be used as a proxy for the past three months of perceived stress, or if the time frames of perceived stress and HCC measurements need to be exactly aligned to examine agreement. It has also been suggested that limitations with perceived stress questionnaires, like the PeSS and PaSS, could affect self-reported stress and reduce agreement with HCC (26). For example, social desirability bias may reduce the reported levels of stress in the parent subgroup because the PaSS includes items specifically related to the challenges of parenting such as 'I enjoy spending time with my child(ren)' and 'If I had to do it over, I might decide not have children' which may not be answered honestly by all parents. It is also possible that individual differences between participants may account for some of the observed disagreement. For example, it has been suggested that exposure to adverse life events or trauma can lead to HPA axis dysfunction and altered cortisol secretion (7,23). This would likely lead to low levels of agreement between perceived stress and HCC in these individuals because of unusually high or low levels of cortisol being released in response to a perceived threat. However, it is unknown how many participants in this sample may have been exposed to adverse life events or trauma or if individuals have altered HPA axis responses.

Beyond the overall lack of agreement that was observed between HCC and perceived stress, a pattern was observed in the Bland-Altman plot of the youth subgroup, where at high and low levels of stress, hair cortisol concentrations were higher relative to PeSS scores. Conversely, at moderate levels of stress, PeSS score were higher than HCC. A similar pattern was observed in the parent subgroup, however, only at low levels of stress. The high HCC, relative to perceived stress, at lower levels of stress could indicate a baseline level of cortisol secretion even when the individual is not under stress. Although cortisol plays an important role in the stress response, a basal level of cortisol is also secreted under non-stressful conditions and works to regulate several processes including blood pressure, inflammatory and immune responses, and fat and glucose metabolism (18,19). This means that even when no stressors are present and an individual reports little to no perceived stress, some cortisol is still released and therefore a higher HCC would be expected under these conditions, relative to perceived stress. It is currently unclear what is causing the other patterns in the youth subgroup (i.e., higher HCC at high levels of stress and higher PeSS scores at moderate levels of stress). It is possible that some of these patterns of disagreement could be driven by individual's with HPA axis dysfunction, who have under- or over-active cortisol responses. As previously discussed, this may cause an unusually high or low level of cortisol to be released in response to a perceived threat, causing disagreement between HCC and perceived stress. However, this notion is speculative, and it is unknown if the youth in this sample had HPA axis dysfunction, or if something else was driving these patterns.

This exploratory study was the first to directly examine agreement between HCC and perceived stress and use Bland-Altman plots to visually examine the patterns of agreement. The low levels of agreement observed between perceived stress and HCC in both subgroups provides preliminary evidence that both measures should be used to comprehensively assess the stress response, as single measures of either perceived stress or HCC may under- or over-estimate stress levels. Future studies are needed to confirm the observed patterns of agreement and to further explore what is driving these patterns if they are observed.

5.2 Objective 2 – Risk factors for psychological and physiological stress

The hypothesis that sociodemographic and psychosocial variables would be associated with both perceived stress and HCC in the youth and parent subgroups was partially supported.

Age was not significantly associated with perceived stress or HCC in either the youth or parent subgroups. The lack of association observed between age and each measure of stress is likely due to the narrow age ranges included in each subgroup. The youth subgroup only included adolescents aged 14-17 and the parent subgroup ranged from 33-65 years. This may have prevented any effect of age from being observed, particularly in the youth subgroup. Most previous studies which have observed an effect of age on perceived stress or HCC included participants from a larger age range, and typically found significant differences between younger adults or children and older adults, but no differences within these age groups (33,46–50).

Female sex was associated with higher perceived stress scores in the youth subgroup and lower HCC in the parent subgroup. Both of these findings align with previous literature as females typically report higher levels of perceived stress (54–58), and have lower HCC than males (26,33,51–53,59–66). However, these results were not found in both subgroups (i.e., female sex was not associated with HCC in youth or perceived stress score in parents). It is possible that sex was not associated with perceived stress in the parent subgroup because all parents included in this study were the primary caregiver for their child. A previous study examining depressive symptoms in parents of children with epilepsy suggested that the parent being the primary caregiver for the child may be a more important determinant of depressive symptoms than whether the parent is a mother or a father (137). This notion may also apply to a parent's perceived stress and therefore, because all parents in this study were the primary caregiver for their child, and the measure of perceived stress in this group specifically asked about parenting stress, the sex of the parent may not have made a significant difference in their perceived stress. The lack of association found between sex and HCC in the youth subgroup may be explained by the age of participants. It has been suggested that the differences in HCC between males and females may be due to biological differences between the sexes, resulting in a higher level of total cortisol levels in males (52). Because of the relatively young age of the youth in this sample, it is possible that these biological differences may not be fully developed during adolescence and therefore no association between sex and HCC could be observed. Previous studies have found boys to have higher HCC than girls in populations of younger children (<10 years) (53,59,60,62,63,66), however in samples of adolescents similar in age to the youth included in this study, results have been mixed, with studies commonly finding higher HCC in girls (24,67) or no association (42,49,50,62,84). Future studies should focus on adolescents to determine if the sex differences in HCC typically observed in other age groups also applies to this group, or if adolescence is a developmentally distinct period where these differences are not observed.

Having an annual household income of <$90,000 was associated with higher HCC in the youth subgroup. This finding is consistent with most previous studies examining the relationship between HCC and household income in children and adolescents (53,67,68). This relationship between lower income and increased HCC is typically thought to be related to more stressful life circumstances associated with lower income (53). Some studies have pointed to other factors related to socioeconomic status such as parental education or minority status, that may also influence HCC in a similar way (53), but due to the limited number of variables that were able to be included in the models, these factors were unable to be examined. One previous study did find a positive association between income and HCC in children; however, this study included a large proportion of children who had experienced maltreatment and had a reduction in HCC which may have influenced the observed association with income and the different findings to this study (50). Lower income, however, was not found to be a significant predictor of HCC in the parent subgroup or of perceived stress in either subgroup.

Higher parent psychopathology scores were found to be associated with increased HCC and perceived stress in the youth subgroup. Although few previous studies have examined the relationship between a parent's psychopathology and their child's perceived stress and/or HCC, these results are not unexpected. A previous study also found a positive relationship between parents' psychopathology and their child's HCC and suggested that this HPA axis dysfunction may be due to environmental changes due to parental mood (75). No previous studies have explored this association with perceived stress, but the effects of parent psychopathology on the child's environment is likely to influence perceived stress in a similar way.

In the youth subgroup, the presence of a chronic physical illness was associated with significantly lower perceived stress scores. This finding was unexpected as worsening symptoms of physical illnesses have previously been reported to be associated with perceived stress (70–72). However, none of these studies considered the association between perceived stress and chronic physical illness in a clinical sample of individuals who all have a comorbid mental disorder. This study specifically asked about physical illnesses that were diagnosed prior to mental disorder. A diagnosis of physical illness can place strain on both youth and their families (138,139). In response to this, many youth and families develop adaptive coping strategies in order to manage these strains and reduce stress (139,140). Therefore, when these youth face subsequent mental illness, they may already have effective coping strategies and resources in place, such as a greater ability to navigate the healthcare system (111), to better cope with their mental disorder. These strategies may then reduce the extent of perceived stress these youth experience (138,139), compared to youth with mental disorder who have not experienced a previous physical illness, and therefore not yet developed appropriate coping mechanisms. However, this relationship has never been directly assessed and future studies should be conducted to determine if previously diagnosed physical illnesses do elicit adaptive coping that reduce perceived stress in youth with mental disorder. It was also unexpected that the presence of a chronic physical illness was not significantly associated with HCC. Previous studies have found that individuals with physical illnesses typically have elevated HCC (31,33,48,52,65,73,74). However, most of these studies have observed this relationship in adults with illnesses such as heart disease and diabetes, whereas this sample only included youth and the majority of reported physical illnesses were asthma, which could account for the different findings.

In the parent subgroup, their child's age, sex, and whether their child had a chronic physical illness were not associated with the parent's HCC or perceived stress score. These findings indicate that characteristics of their child may not influence a parent's psychological or physiological stress. This is somewhat unexpected, particularly for perceived stress, because parent's perceived stress was measures using the Parental Stress Scale, which focuses on stress specifically relating to their role as a parent. It would therefore be expected that certain characteristics of their child, such as their child having a chronic physical illness, may influence their feelings of stress. For example, as previously discussed, families often develop adaptive coping strategies in response to their child being diagnosed with a physical illness (139,140), which may be utilized after a subsequent diagnosis of mental illness, and reduce the stress experienced by the family (138,139). However, these results suggest that such factors may affect the child's stress more than the parent's stress. No previous studies have explored the relationship between parent's perceived stress or HCC and characteristics of their child and therefore studies with a more diverse population of children across a broader age range, a more even proportion of males and females, and including children with more diverse chronic physical illnesses are needed to confirm these findings.

Higher family functioning scores were associated with higher levels of perceived stress in the parent subgroup. No studies have previously assessed the relationship between family functioning and perceived stress or HCC in parents. It is possible that because this sample was comprised of parents of children receiving mental health treatments that having a higher degree of family functioning may have made them more aware of their child's illness and increased their stress levels, particularly because the measure used to capture perceived stress in this subgroup was specifically related to parenting stress. The McMaster Family Assessment Device, which was used to measure family functioning, captures elements of communication, affective involvement, and affective responsiveness, among others (126). Therefore, higher scores on this scale may indicate that a parent would have more awareness of their child's disorder and symptoms and increase their levels of parenting stress. A previous study found that higher levels of affective involvement in parents was associated with increased odds of major depressive disorder in their children (129). It was suggested that children with major depressive disorder may need greater support from parents to manage interpersonal situations and lead to greater affective involvement by parents, however, the level of support required to support their child may also contribute to greater conflict and emotional withdrawal in the parent (129). Therefore, greater levels of affective involvement by parents in this sample, who all have children with mental disorder, could also lead to greater levels of parenting stress brought on by the high level of support they are providing to their children. However, this notion is speculative and future studies should further explore the impacts of family functioning on parent's perceived stress, particularly in parent's who have children with mental disorders.

The hypothesis that the directionality or magnitude of the associations between potential risk factors and perceived stress and HCC, respectively, may be different was supported. The MOVER analysis comparing the strength of the associations between each of the covariates and HCC/perceived stress score indicated that when there was a significant difference in a covariate's associations with HCC and perceived stress score, they were more strongly associated with perceived stress score than HCC. In the youth subgroup, female sex was significantly associated with PeSS score, but not HCC and when the strength of association was compared between the two measures, it was found that female sex had a stronger association with PeSS score than HCC. The same was found for family functioning in the parent subgroup. Additionally, both household income in the youth subgroup and female sex in the parent subgroup were significantly associated with HCC and not associated with perceived stress. However, the MOVER analysis indicated that the strength of these associations was not significantly different between HCC and perceived stress in either subgroup. These results all indicate that sociodemographic and psychosocial factors may have a stronger association with perceived stress than with HCC. This provides further evidence that there is a lack of psychoendocrine covariance between measures of psychological and physiological stress as certain risk factors may affect the psychological and physiological stress responses differently.

5.3 Objective 3 – Association between stress and mental disorder

The hypothesis that perceived stress would be positively associated with all included mental disorders was partially supported. In the parent subgroup, higher PaSS scores were significantly associated with higher scores on both the CES-D and STAI. However, in the youth subgroup, higher PeSS scores were associated with an increased odds of the presence of major depressive disorder, generalized anxiety, and separation anxiety, but not with phobia, attention-deficit hyperactivity disorder, or oppositional defiant/conduct disorder. The observed associations between higher levels of perceived stress and mental disorder aligns with previous studies that have found that psychological stress is associated with both depression and anxiety (34,37,42,54,76,98,99,103). Perceived stress and the symptoms of internalizing disorders are likely related as increased levels of stress are related to an perceived inability to cope, feeling overwhelemed, etc., which are also commonly associated with symptoms of depression and anxiety (76,99). The lack of association found between perceived stress and attention-deficit hyperactivity disoder does not align with previous studies which have found associations with higher levels of perceived stress (100–102). Similarly, while no studies have previously examined the association between perceived stress and oppositional defiant disorder, conduct disorder, or phobia, these disorders were expected to follow the same pattern as other mental disorders and be associated with higher levels of perveived stress. The lack of association observed between perceived stress and these disorders could be because more specific disorder types could not be included. For example, there is some evidence that within attention-deficit hyperactivity disorder, inattentive symptoms may be more closely related to perceived stress than hyperactivity symptoms (100,102). Due to the sample size available in this study, the individual types of attention-deficit hyperactivity disorder could not be analyzed separately and only overall attention-deficit hyperactivity disorder was included. Similarly, social phobia and specific phobia, and oppositional defiant disorder and conduct disorder were analyzed together, which may have affected the observed associations. Future studies should evaluate each of these disorders independently to determine if the lack of association obsevred in this sample was due to similar disorders being grouped together, or if there is no true association with perceived stress.

The hypothesis that HCC would be associated with mental disorders, but that the directionality may differ between disorders was partially supported. In the youth subgroup, higher HCC was associated with increased odds of generalized anxiety, and not associated with any other disorders. In the parent subgroup higher HCC was associated with higher CES-D scores but was not associated with STAI scores. The finding that higher HCC was associated with increased odds of generalized anxiety in the youth subgroup was unexpected as previous studies have typically observed lower HCC in individuals with generalized anxiety disorder (51,67,108). This finding may be due to the age of this sample, including youth aged 14-17. The hypocortisolism typically observed in patients with generalized anxiety disorder is thought to be a result of attenuation of the stress response over time after an initial increase in cortisol release (67,108). Because of the relatively young age of the participants included in this sample, they may still be experiencing the initial increase in cortisol secretion, and not yet attenuated to this response (141). This aligns with recent findings that patients with shorter duration anxiety disorders have elevated HCC, while patients with longer durations illness do not (106). Lower HCC has previously been found to be associated with anxiety symptoms in youth (67), however, that study used a sample of healthy youth, as opposed to the clinical sample of youth receiving mental health treatment in the current study. Future studies in clinical samples of youth are needed to confirm the finding of elevated HCC in patients with generalized anxiety disorder, and longitudinal studies are needed to determine if this initial increase in cortisol attenuates over time. There was no association found between HCC and anxiety symptoms in the parent subgroup. This may be because the STAI was used, which assesses general anxiety symptoms and was not screening for generalized anxiety disorder, as in the youth subgroup. Other anxiety-related disorders (separation anxiety and phobia) were also not associated with HCC in the youth subgroup, indicating that it may be generalized anxiety disorder specifically which is associated with

HCC, and not anxiety symptoms or related disorders. It is also possible because the parent subgroup was not a clinical sample, their symptoms may not have been severe enough to be significantly associated with HCC.

The positive association between HCC and CES-D scores in the parent subgroup aligns with previous studies which have found increased HCC to be associated with depression (28,67,104,105) and provides additional evidence that depression is associated with HPA axis dysfunction. However, no association was found between major depressive disorder and HCC in the youth subgroup. There is some emerging evidence that the association between depression and HCC may be curvilinear, with both high and low levels of cortisol being associated with depression (109). This relationship is plausible as it aligns with the thought that HPA axis dysfunction manifests as both under- and over-activation of the cortisol response. However, because this analysis used binary logistic regression to measure the association between HCC and major depressive disorder, a curvilinear relationship would not be able to be detected, and it is possible that this is masking the true association. However, it is unclear if this is happening in this sample, or if there is truly no association between HCC and major depressive disorder. Future studies should consider evaluating the association between HCC and depression in a curvilinear fashion to determine if the disorder is associated with both high and low cortisol levels.

It was unexpected that generalized anxiety disorder was the only disorder associated with HCC in the youth subgroup. However, while many studies have observed significant associations between HCC and various mental disorders, many others have also failed to observe significant associations (39,42,45,51,60,84,90,106). The youth subgroup was a comprised of a clinical sample of youth receiving mental health treatment, and almost all youth screened positive for two or more mental disorders. The high rate of comorbidity between mental disorders in this sample may have affected the observed associations with HCC. For example, there is some preliminary evidence that the co-occurrence of major depressive disorder and generalized anxiety disorder may result in different cortisol patterns than the presence of each disorder individually (37). However, no studies have evaluated the effect of comorbidity of other disorders included in this analysis, such as attention-deficit hyperactivity disorder, or the effect of comorbidity of more than two disorders.

Overall, these results indicate that both measures of psychological and physiological stress may be associated with several mental disorders, particularly depression and anxiety, however they may each have different relationships with different disorders. This aligns with the results of the previous two objectives in this sample that indicated that measures of perceived stress and HCC had low agreement and different associations with various demographic and psychosocial risk factors in this sample. Though this study did not find significant associations between perceived stress or HCC and externalizing disorders or phobia, future studies should continue to explore these associations with larger samples and account for the effects of comorbidity, particularly in clinical samples.

5.4 Limitations

An important limitation of this study was the limited sample size available for these analyses, particularly for the youth subgroup as only youth aged 14-17 completed the PeSS. Because of the limited sample size only a small number of potential risk factors for high levels of perceived stress or HCC could be examined for the second objective. Some potentially relevant covariates such as quality of life or disability could not be included in the models. Additionally, when examining the association between specific mental disorders and perceived stress/HCC in the youth subgroup, the effects of comorbidity could not be assessed because of the limited sample size and therefore the effects of comorbidity of mental disorders on the observed associations in this sample are unknown. Similarly, because this study was a secondary data analysis, only variables that were collected as part of the original study could be analysed. Therefore, variables of interest such as exposure to adverse life events or trauma, which may have had an important effect on HCC (7,23) and mental health (142,143), could not be included and the effects of these variables are unknown.

Additionally, the generalizability of this study is limited because of the relatively narrow population included. All youth eligible for inclusion were receiving mental health services from a single centre and all parents had children receiving these services. Most participants included in this study were also Caucasian. As ethnicity and minority status have been suggested to be related to HCC and HPA axis dysfunction (53), it is possible that a primarily Caucasian sample may also limit the generalizability of these results. Although the results of this study are valuable as the first analysis directly assessing the agreement between perceived stress and HCC, and their relative associations with mental disorder in a clinical population of youth and their parents, they are likely not generalizable to a broader population.

Another limitation is that in that parent subgroup, perceived stress was measured using the Parental Stress Scale, which specifically asks respondents about stress related to parenting (113). While the PaSS has been shown to be positively correlated with other measures of perceived stress, such as the Perceived Stress Scale, these correlations were moderate in size (r=0.41 to 0.53) (113). Therefore, it is likely that while the PaSS captures stress related to parenting, it does not capture the individual's overall perceived stress. This may have impacted some of the results in the parent subgroup. For example, the ICC in the parent subgroup was lower than in the youth subgroup (0.15 vs. 0.31, respectively), which may represent that parenting stress is less related to HCC than overall perceived stress. Future studies should use a general measure of perceived stress, such as the Perceived Stress Scale, to measure the agreement between perceived stress and HCC in this population to determine if using a measure of parenting stress impacted the observed agreement in this study, or if it remains low.

Finally, because the data used in this study was cross-sectional, the directionally of the observed associations cannot be established. Particularly for the analyses in objective 3, examining the association between perceived stress and HCC and mental disorder, it is unclear if increased levels of psychological or physiological stress are leading to the increased odds of mental disorders, or if the presence of a mental disorder is increasing psychological or physiological stress. Future studies with a longitudinal design are needed to examine the directionality of these associations.

5.5 Implications & Future Directions

The primary implication of this work is that it provides preliminary evidence that the agreement between perceived stress and HCC is low. These results indicate that these measures should not be used as clinical correlates of one another and that both are needed to comprehensively evaluate the stress response. It is currently unclear whether the low agreement observed in this study is due to limitations of the measures such as different time periods being captured between the measures or exposure to adversity causing HPA axis dysfunction in some individuals, or if it indicates a true lack of psychoendocrine covariance between the psychological and physiological stress response. However, until this can be determined both measures should continue to be used to ensure the comprehensive assessment of the stress response. Future studies should continue to explore the agreement between psychological and physiological measures of stress to confirm the low level of agreement found in this study and explore potential factors that may be contributing to the lack of agreement such as exposure to adverse life events.

It is also important for clinicians to consider that agreement between the psychological and physiological stress responses may be low and that the level of psychological stress conveyed by a patient may not match the physiological response. Because it is unlikely that clinicians will directly assess the physiological stress response, understanding that there may be a lack of psychoendocrine covariance and that assessing psychological stress may not accurately represent the full extent of that individual's stress response is important. This also highlights the need to further research into how factors such as exposure to early life adversity or trauma affect HPA axis dysfunction and if this is related to the low agreement between perceived stress and HCC. If exposure to these experiences, or other related factors, cause HPA axis dysfunction and this drives the lack of psychoendocrine covariance between psychological and physiological stress in these individuals, it would be important for clinicians to identify these individuals and understand that their level of psychological stress is likely not indicative of their physiological response.

The results of this study also suggest that sociodemographic and psychosocial factors may be more closely associated with perceived stress and with HCC. It is therefore also important that clinicians also consider that certain demographic or psychosocial variables such as sex or the presence of a chronic physical illness may be influencing psychological stress more than physiological stress. This is particularly relevant in the context of the observed low agreement between perceived stress and HCC because these factors may be affecting the psychological stress a patient is conveying more than their physiological response. Future studies are needed to continue to explore the relationship between the risk factors and psychological and physiological stress in using larger and more diverse to confirm the preliminary results found in this study and elucidate the relationship between these factors and both psychological and physiological stress.

These results also indicate that both perceived stress and HCC may be associated with mental disorders, particularly depression and anxiety. However, because of the high prevalence of comorbidity of mental disorders in this sample and the fact that the effects of the comorbidity could not be examined, it remains unclear how these measures of stress are individually related to different mental disorders. This is particularly important for future research to consider as comorbidity of mental disorders is common, particularly in clinical samples of youth (144,145). Future studies need to explore how comorbidity of mental disorders affect their association with both HCC and perceived stress to fully understand the relationship between psychological and physiological stress and mental disorders.

Future research should most importantly continue to explore the agreement between measures of psychological and physiological stress and explore potential reasons for the low level of agreement if this pattern continues to be found. If low agreement continues to be found in larger and more diverse samples, it is then important to elucidate the sociodemographic and psychosocial factors that influence measures of psychological and physiological and how each measure is associated with various mental disorders. If there are different risk factors for psychological and physiological stress and each are associated differently with the presence of mental disorders, it is important for clinicians to be aware of these factors and how the psychological stress communicated by a patient may not reflect their physiological response and may not accurately identify vulnerability for certain mental disorders.

CONCLUSION

The results of this study provide preliminary evidence that agreement between measures of psychological and physiological stress is low in a clinical sample of youth and their parents. It was also identified that sociodemographic and psychosocial factors may be associated with both psychological and physiological stress, although they may have different relationships with each measure, and various mental disorders may also be associated with each measure of stress. Together these results indicate that there may be a lack of psychoendocrine covariance between measures of psychological and physiological stress, although the reason for this lack of agreement is not yet clear. It is suggested that both measures should be used to comprehensively evaluate the stress response and that it is particularly important for clinicians to be aware of the apparent lack of psychoendocrine covariance in cases where physiological stress cannot be examined. Further investigation into the relationship between psychological and physiological stress is needed in larger and more diverse samples to confirm the findings of this study and to continue to examine factors associated with psychological and physiological stress and how these measures are related to mental disorders.

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APPENDIX A – Perceived Stress Scale

The questions in this section ask you about your feelings and thoughts during the last month. In each case, please circle the answer that best describes how often you felt or thought a certain way.

		Never	Almost Never	Sometimes	Fairly Often	Very Often
a.	In the last month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
b.	In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
c.	In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
d.	In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
e.	In the last month, how often have you felt that things were going your way?	0	1	2	3	4
f.	In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
g.	In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
h.	In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
i.	In the last month, how often have you been angered because of things that were outside of your control?	0	1	2	3	4
j.	In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

APPENDIX B – Parental Stress Scale

The questions in this section ask you about your feelings and thoughts as a parent during the past month.

		Strongly Disagree	Disagree	Neither Disagree/Agree	Agree	Strongly Agree
a.	I am happy in my role as a parent.	01	02	03	04	05
b.	There is little or nothing I wouldn't do for my child(ren) if it was necessary.	01	02	03	04	05
c.	Caring for my child(ren) sometimes takes more time and energy than I have to give.	01	02	03	04	05
d.	I sometimes worry whether I am doing enough for my child(ren).	01	02	03	04	05
e.	I feel close to my child(ren).	01	02	03	04	05
f.	I enjoy spending time with my child(ren).	01	02	03	04	05
g.	My child(ren) is (are) an important source of affection for me.	01	02	03	04	05
h.	Having children gives me a more certain and optimistic view for the future.	01	02	03	04	05
i.	The major source of stress in my life is my child(ren).	01	02	03	04	05
j.	Having children leaves little time and flexibility in my life.	01	02	03	04	05
k.	Having children has been a financial burden.	01	02	03	04	05
l.	It is difficult to balance different responsibilities because of my child(ren).	01	02	03	04	05
m.	The behaviour of my child(ren) is often embarrassing or stressful to me.	01	02	03	04	05
n.	If I had it to do over, I might decide not to have children.	01	02	03	04	05
o.	I feel overwhelmed by the responsibility of being a parent.	01	02	03	04	05
p.	Having children has meant having too few choices and too little control over my life.	01	02	03	04	05
q.	I am satisfied as a parent.	01	02	03	04	05
r.	I find my child(ren) enjoyable.	01	02	03	04	05

APPENDIX C – OBJECTIVE 2 HYPOTHESIZED MODELS

Youth Subgroup:

Model 1: Youth perceived stress (block 1)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i$$
$$PeSS_i = \beta_0 + \beta_1 YouthAge_i + \beta_2 YouthSex_i + \beta_3 Income_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for Perceived Stress Scale score of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{2i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for household income (0 < \$90,000/year, 1 ≥ \$90,000/year) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to youth age,
β_2 is the fixed unknown regression coefficient corresponding to youth sex,
β_3 is the fixed unknown regression coefficient corresponding to household income,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j$, $(X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 2: Youth perceived stress (block 2)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \beta_5 X_{5i} + \varepsilon_i$$
$$PeSS_i = \beta_0 + \beta_1 YouthAge_i + \beta_2 YouthSex_i + \beta_3 Income_i + \beta_4 ParentPsychopathology_i + \beta_5 CPI_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for Perceived Stress Scale score of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{2i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for household income (0 < \$90,000/year, 1 ≥ \$90,000/year) of the *i*-th subject,
X_{4i} is the observed continuous predictor variable for parent psychopathology (combined CESD/STAI score) of the *i*-th subject,
X_{5i} is the observed binary predictor variable for chronic physical illness (0=no, 1=yes) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to youth age,
β_2 is the fixed unknown regression coefficient corresponding to youth sex,
β_3 is the fixed unknown regression coefficient corresponding to household income,
β_4 is the fixed unknown regression coefficient corresponding to parent psychopathology,
β_5 is the fixed unknown regression coefficient corresponding to chronic physical illness,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j$, $(X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 3: Youth HCC (block 1)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i$$
$$HCC_i = \beta_0 + \beta_1 YouthAge_i + \beta_2 YouthSex_i + \beta_3 Income_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for youth HCC of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{2i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for household income (0 < \$90,000/year, 1 ≥ \$90,000/year) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to youth age,
β_2 is the fixed unknown regression coefficient corresponding to youth sex,
β_3 is the fixed unknown regression coefficient corresponding to household income,

ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j$, $(X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 4: Youth HCC (block 2)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \beta_5 X_{5i} + \varepsilon_i$$
$$HCC_i = \beta_0 + \beta_1 YouthAge_i + \beta_2 YouthSex_i + \beta_3 Income_i + \beta_4 ParentPsychopathology_i + \beta_5 CPI_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for youth HCC of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{2i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for household income (0 < \$90,000/year, 1 ≥ \$90,000/year) of the *i*-th subject,
X_{4i} is the observed continuous predictor variable for parent psychopathology (combined CESD/STAI score) of the *i*-th subject,
X_{5i} is the observed binary predictor variable for chronic physical illness (0=no, 1=yes) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to youth age,
β_2 is the fixed unknown regression coefficient corresponding to youth sex,
β_3 is the fixed unknown regression coefficient corresponding to household income,
β_4 is the fixed unknown regression coefficient corresponding to parent psychopathology,
β_5 is the fixed unknown regression coefficient corresponding to chronic physical illness,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j$, $(X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

<u>Parent Subgroup:</u>

Model 5: Parent perceived stress (block 1)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i$$
$$PaSS_i = \beta_0 + \beta_1 ParentAge_i + \beta_2 ParentSex_i + \beta_3 Income_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for Parental Stress Scale score of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for parent age (in years) of the *i*-th subject,
X_{2i} is the observed binary predictor variable for parent sex (0=female, 1=male) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for household income (0 < \$90,000/year, 1 ≥ \$90,000/year) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to parent age,
β_2 is the fixed unknown regression coefficient corresponding to parent sex,
β_3 is the fixed unknown regression coefficient corresponding to household income,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j$, $(X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 6: Parent perceived stress (block 2)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \beta_5 X_{5i} + \beta_6 X_{6i} + \varepsilon_i$$
$$PaSS_i = \beta_0 + \beta_1 ParentAge_i + \beta_2 ParentSex_i + \beta_3 Income_i + \beta_4 YouthAge_i + \beta_5 YouthSex_i + \beta_6 YouthCPI_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for Parental Stress Scale score of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for parent age (in years) of the *i*-th subject,
X_{2i} is the observed binary predictor variable for parent sex (0=female, 1=male) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for household income (0 < \$90,000/year, 1 ≥ \$90,000/year) of the *i*-th subject,
X_{4i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{5i} is the observed continuous predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,

X_{6i} is the observed binary predictor variable for youth chronic physical illness (0=no, 1=yes) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to parent age,
β_2 is the fixed unknown regression coefficient corresponding to parent sex,
β_3 is the fixed unknown regression coefficient corresponding to household income,
β_4 is the fixed unknown regression coefficient corresponding to youth age,
β_5 is the fixed unknown regression coefficient corresponding to youth sex,
β_6 is the fixed unknown regression coefficient corresponding to youth chronic physical illness,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0,\sigma^2)$, for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 7: Parent perceived stress (block 3)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \beta_5 X_{5i} + \beta_6 X_{6i} + \beta_7 X_{7i} + \varepsilon_i$$

$$PaSS_i = \beta_0 + \beta_1 ParentAge_i + \beta_2 ParentSex_i + \beta_3 Income_i + \beta_4 YouthAge_i + \beta_5 YouthSex_i + \beta_6 YouthCPI_i + \beta_7 FAD_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for Parental Stress Scale score of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for parent age (in years) of the *i*-th subject,
X_{2i} is the observed binary predictor variable for parent sex (0=female, 1=male) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for household income (0 < \$90,000/year, 1 ≥ \$90,000/year) of the *i*-th subject,
X_{4i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{5i} is the observed continuous predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
X_{6i} is the observed binary predictor variable for youth chronic physical illness (0=no, 1=yes) of the *i*-th subject,
X_{7i} is the observed continuous predictor variable for family functioning (McMaster Family Assessment Device score) or the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to parent age,
β_2 is the fixed unknown regression coefficient corresponding to parent sex,
β_3 is the fixed unknown regression coefficient corresponding to household income,
β_4 is the fixed unknown regression coefficient corresponding to youth age,
β_5 is the fixed unknown regression coefficient corresponding to youth sex,
β_6 is the fixed unknown regression coefficient corresponding to youth chronic physical illness,
β_7 is the fixed unknown regression coefficient corresponding to family functioning,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0,\sigma^2)$, for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 8: Parent HCC (block 1)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i$$

$$ParentHCC_i = \beta_0 + \beta_1 ParentAge_i + \beta_2 ParentSex_i + \beta_3 Income_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for parent HCC of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for parent age (in years) of the *i*-th subject,
X_{2i} is the observed binary predictor variable for parent sex (0=female, 1=male) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for household income (0 < \$90,000/year, 1 ≥ \$90,000/year) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to parent age,
β_2 is the fixed unknown regression coefficient corresponding to parent sex,
β_3 is the fixed unknown regression coefficient corresponding to household income,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0,\sigma^2)$, for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 9: Parent HCC (block 2)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \beta_5 X_{5i} + \beta_6 X_{6i} + \varepsilon_i$$

$$ParentHCC_i = \beta_0 + \beta_1 ParentAge_i + \beta_2 ParentSex_i + \beta_3 Income_i + \beta_4 YouthAge_i + \beta_5 YouthSex_i + \beta_6 CPI_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for parent HCC of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for parent age (in years) of the *i*-th subject,
X_{2i} is the observed binary predictor variable for parent sex (0=female, 1=male) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for household income (0 < \$90,000/year, 1 ≥ \$90,000/year) of the *i*-th subject,
X_{4i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{5i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{6i} is the observed binary predictor variable for youth chronic physical illness (0=no, 1=yes) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to parent age,
β_2 is the fixed unknown regression coefficient corresponding to parent sex,
β_3 is the fixed unknown regression coefficient corresponding to household income,
β_4 is the fixed unknown regression coefficient corresponding to youth age,
β_5 is the fixed unknown regression coefficient corresponding to youth sex,
β_6 is the fixed unknown regression coefficient corresponding to youth chronic physical illness,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j$, $(X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 10: Parent HCC (block 3)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + \beta_5 X_{5i} + \beta_6 X_{6i} + \beta_7 X_{7i} + \varepsilon_i$$

$$ParentHCC_i = \beta_0 + \beta_1 ParentAge_i + \beta_2 ParentSex_i + \beta_3 Income_i + \beta_4 YouthAge_i + \beta_5 YouthSex_i + \beta_6 YouthCPI_i + \beta_7 FAD_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for parent HCC of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for parent age (in years) of the *i*-th subject,
X_{2i} is the observed binary predictor variable for parent sex (0=female, 1=male) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for household income (0 < \$90,000/year, 1 ≥ \$90,000/year) of the *i*-th subject,
X_{4i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{5i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{6i} is the observed binary predictor variable for youth chronic physical illness (0=no, 1=yes) of the *i*-th subject,
X_{7i} is the observed continuous predictor variable for family functioning (McMaster Family Assessment Device score) or the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to parent age,
β_2 is the fixed unknown regression coefficient corresponding to parent sex,
β_3 is the fixed unknown regression coefficient corresponding to household income,
β_4 is the fixed unknown regression coefficient corresponding to youth age,
β_5 is the fixed unknown regression coefficient corresponding to youth sex,
β_6 is the fixed unknown regression coefficient corresponding to youth chronic physical illness,
β_7 is the fixed unknown regression coefficient corresponding to family functioning,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j$, $(X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

APPENDIX D – OBJECTIVE 3 HYPOTHESIZED MODELS

<u>Youth Subgroup</u>:

Model 1: Youth perceived stress (MDD)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$MDD_i = \beta_0 + \beta_1 PeSS_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of MDD for the *i*-th subject,

Y_i is the unknown binary outcome variable for MDD (0=No MDD, 1=MDD) of the *i*-th subject;
X_{1i} is the observed continuous predictor variable for Perceived Stress Scale score of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 2: Youth HCC (MDD)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$MDD_i = \beta_0 + \beta_1 YouthHCC_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of MDD for the *i*-th subject,

Y_i is the unknown binary outcome variable for MDD (0=No MDD, 1=MDD) of the *i*-th subject;
X_{1i} is the observed continuous predictor variable for youth HCC of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 3: Youth perceived stress (GAD)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$GAD_i = \beta_0 + \beta_1 PeSS_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of GAD for the *i*-th subject,

Y_i is the unknown binary outcome variable for GAD (0=No GAD, 1=GAD) of the *i*-th subject;
X_{1i} is the observed continuous predictor variable for Perceived Stress Scale score of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 4: Youth HCC (GAD)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$GAD_i = \beta_0 + \beta_1 YouthHCC_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of GAD for the *i*-th subject,

Y_i is the unknown binary outcome variable for GAD (0=No GAD, 1=GAD) of the *i*-th subject;

X_{1i} is the observed continuous predictor variable for youth GAD of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 5: Youth perceived stress (ADHD)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$ADHD_i = \beta_0 + \beta_1 PeSS_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of ADHD for the *i*-th subject,

Y_i is the unknown binary outcome variable for ADHD (0=No ADHD, 1=ADHD) of the *i*-th subject;

X_{1i} is the observed continuous predictor variable for Perceived Stress Scale score of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 6: Youth HCC (ADHD)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$ADHD_i = \beta_0 + \beta_1 YouthHCC_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of ADHD for the *i*-th subject,

Y_i is the unknown binary outcome variable for ADHD (0=No ADHD, 1=ADHD) of the *i*-th subject;

X_{1i} is the observed continuous predictor variable for youth HCC of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,

β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 7: Youth perceived stress (ODD/CD)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$ODD/CD_i = \beta_0 + \beta_1 PeSS_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of ODD/CD for the *i*-th subject,

Y_i is the unknown binary outcome variable for ODD/CD (0=No ODD/CD, 1=ODD/CD) of the *i*-th subject;

X_{1i} is the observed continuous predictor variable for Perceived Stress Scale score of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 8: Youth HCC (ODD/CD)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$ODD/CD_i = \beta_0 + \beta_1 YouthHCC_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of ODD/CD for the *i*-th subject,

Y_i is the unknown binary outcome variable for ODD/CD (0=No ODD/CD, 1=ODD/CD) of the *i*-th subject;

X_{1i} is the observed continuous predictor variable for youth HCC of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 9: Youth perceived stress (phobia)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$Phobia_i = \beta_0 + \beta_1 PeSS_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of phobia for the *i*-th subject,

Y_i is the unknown binary outcome variable for phobia (0=No phobia, 1=phobia) of the *i*-th subject;

X_{1i} is the observed continuous predictor variable for Perceived Stress Scale score of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 10: Youth HCC (phobia)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$Phobia_i = \beta_0 + \beta_1 YouthHCC_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of phobia for the *i*-th subject,

Y_i is the unknown binary outcome variable for phobia (0=No phobia, 1=phobia) of the *i*-th subject;

X_{1i} is the observed continuous predictor variable for youth HCC of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 11: Youth perceived stress (separation anxiety)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$
$$SepAnx_i = \beta_0 + \beta_1 PeSS_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of separation anxiety for the *i*-th subject,

Y_i is the unknown binary outcome variable for separation anxiety (0=No separation anxiety, 1=separation anxiety) of the *i*-th subject;

X_{1i} is the observed continuous predictor variable for Perceived Stress Scale score of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

Model 12: Youth HCC (separation anxiety)

$$\eta_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i}$$

$$SepAnx_i = \beta_0 + \beta_1 YouthHCC_i + \beta_2 YouthAge_i + \beta_3 YouthSex_i$$

Where:

$\eta_i = logit\ (P_r(Y_i = 1)) = \frac{\log(P_r(Y_i=1))}{(1-P_r(Y_i=1)}$ is the log odds of separation anxiety for the *i*-th subject,

Y_i is the unknown binary outcome variable for separation anxiety (0=No separation anxiety, 1=separation anxiety) of the *i*-th subject;

X_{1i} is the observed continuous predictor variable for youth HCC of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for youth age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for youth sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to PeSS score,
β_2 is the fixed unknown regression coefficient corresponding to youth age,
β_3 is the fixed unknown regression coefficient corresponding to youth sex,

Assumption: for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$

<u>Parent Subgroup</u>:

Model 13: Parent perceived stress (CES-D)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i$$
$$CESD_i = \beta_0 + \beta_1 PaSS_i + \beta_2 ParentAge_i + \beta_3 ParentSex_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for CES-D score of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for Parental Stress Scale score of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for parent age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for parent sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to parent CES-D score,
β_2 is the fixed unknown regression coefficient corresponding to parent age,
β_3 is the fixed unknown regression coefficient corresponding to parent sex,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 14: Parent HCC (CES-D)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i$$
$$CESD_i = \beta_0 + \beta_1 ParentHCC_i + \beta_2 ParentAge_i + \beta_3 ParentSex_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for CES-D score of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for parent HCC of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for parent age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for parent sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to parent CES-D score,
β_2 is the fixed unknown regression coefficient corresponding to parent age,
β_3 is the fixed unknown regression coefficient corresponding to parent sex,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j,\ (X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 15: Parent perceived stress (STAI)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i$$
$$STAI_i = \beta_0 + \beta_1 PaSS_i + \beta_2 ParentAge_i + \beta_3 ParentSex_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for STAI score of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for Parental Stress Scale score of the *i*-th subject,

X_{2i} is the observed continuous predictor variable for parent age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for parent sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to parent STAI score,
β_2 is the fixed unknown regression coefficient corresponding to parent age,
β_3 is the fixed unknown regression coefficient corresponding to parent sex,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j$, $(X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$

Model 16: Parent HCC (STAI)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i$$
$$STAI_i = \beta_0 + \beta_1 ParentHCC_i + \beta_2 ParentAge_i + \beta_3 ParentSex_i + \varepsilon_i$$

Where:

Y_i is the observed continuous outcome variable for STAI score of the *i*-th subject,
X_{1i} is the observed continuous predictor variable for parent HCC of the *i*-th subject,
X_{2i} is the observed continuous predictor variable for parent age (in years) of the *i*-th subject,
X_{3i} is the observed binary predictor variable for parent sex (0=female, 1=male) of the *i*-th subject,
β_0 is the fixed unknown intercept,
β_1 is the fixed unknown regression coefficient corresponding to parent STAI score,
β_2 is the fixed unknown regression coefficient corresponding to parent age,
β_3 is the fixed unknown regression coefficient corresponding to parent sex,
ε_i is the unknown random noise where $\varepsilon_i \overset{iid}{\sim} N(0, \sigma^2)$, for any $i \neq j$, $(X_i, Y_i) \perp (X_j, Y_j)$ and $\varepsilon_i \perp X_1, X_2, X_3$