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Protocol

COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
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Project Title: **Evaluation of the Compensatory Reserve Index and Psychosocial Factors in Pediatric Autonomic Dysfunction**

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I. **Hypotheses and Specific Aims**:

Specific Aims:
1) Evaluate the Compensatory Reserve Index (CRI) (i.e., decrement in CRI from supine to standing position) in youth (12 to 21 years old) with Postural Orthostatic Tachycardia Syndrome (POTS; a type of autonomic dysfunction) compared to youth without POTS.
2) Assess associations between CRI values, hypermobility, and parent- and youth-reported symptoms of POTS and associated functional impairment, to determine the utility of CRI in indexing the severity of Autonomic Dysfunction/POTS.
3) Examine psychosocial factors measured by parent and youth report (i.e., symptoms of anxiety and depression, sleep quality, social connectedness, friendship, family functioning, parent behaviors, and parent mental health) in a representative sample of youth with POTS compared to youth without POTS.

Hypotheses:
1) Participants with POTS will have a greater change (decrease) in CRI from baseline following positional changes (i.e., supine to standing) than participants without POTS.
2) Greater change in CRI and/or lower minimal CRI value will be associated with greater severity of symptoms, as measured by increased frequency of parent- and youth-reported symptoms and greater degree of functional impairment.
3) Participants with POTS will show more psychosocial difficulties than participants without POTS, indexed by scores on parent- and youth-report measures of anxiety, depression, sleep quality, social connectedness, friendship, family functioning, parent behaviors, and parent mental health.

II. **Background and Significance**:

Pediatric Autonomic Dysfunction. Autonomic dysfunction (or dysautonomia) describes several medical conditions linked to the autonomic nervous system. One of these is Postural Orthostatic Tachycardia Syndrome (POTS). In pediatric populations, symptoms of autonomic dysfunction include an increase in heart rate when standing up (tachycardia), dizziness, nausea, vomiting, fatigue, headaches, abdominal discomfort, and sleep disturbances (9,10). POTS is specifically characterized by orthostatic intolerance and a forty beats per minute (bpm) increase in heart rate upon standing. The etiology of POTS is poorly understood, and there is limited literature on the management of POTS in pediatric populations. In the U.S., POTS is estimated to affect 1-3 million people (8, 11). Research to date indicates that individuals diagnosed with POTS are predominately female (87.7%) and Caucasian (85.1 to 94%) (8, 11). According to one source in the U.S., the mean age at presentation is 15.6 years old (11).

Initial presentation of POTS often occurs following prolonged recovery from a viral illness or physical injury (9, 10). POTS has been associated with significant functional disability and psychological distress, oftentimes persisting after the inciting illness resolves. Many physicians currently use a tilt table test and traditional vital signs measurement (e.g., blood pressure) to assess the presence of POTS. However, the tilt table test has notable limitations. For example, tilt table tests and orthostatic vital signs have been shown to lead to different heart rate responses with a standing position, specifically a higher increase in heart rate in the tilt table group (66). In this study by Plash et al. (66), the changes in heart rate in tilt tests and orthostatic vital signs were compared in subjects with autonomic dysfunction and healthy controls; ultimately, orthostatic vital sign measurement (over 10 minutes) was found to be superior to a 10-minute tilt table test in accurately identifying patients with autonomic dysfunction. There was also a higher rate of false positive results in the tilt table group (66). Thus, it is very important to develop more effective and efficient tools to screen for and assess POTS illness severity, and it is essential to improve our understanding of psychosocial factors associated with POTS, as these may serve as risk and/or protective factors in individuals with POTS.

Compensatory Reserve Index. One of the most difficult tasks in clinical medicine is the assessment of decreasing volume status (4). This evaluation is usually made by physical examination and a review of the patient's traditional vital signs: heart rate (HR), blood pressure (BP), respiratory rate, and oxygen saturation. These vital signs are, however, notoriously unreliable (18, 19). As computing power and computational methods improve, it is now evident that current monitoring technology is allowing physiological information to pass by unseen and unharnessed. Many pathological states have been characterized by analysis of simple, continuous physiologic waveforms, and previous studies have demonstrated that photoplethysmogram (PPG) waveforms obtained with a pulse oximeter sensor significantly change with volume loss (20-24). In a series of previous experiments, Dr. Steve Moulton (co-investigator on this protocol) and his colleagues used advanced feature extraction and machine-learning methods to extract information from PPG waveforms generated in a model of human blood loss, resulting in an algorithm called the *Compensatory Reserve Index (CRI)* (e.g., 31).

The ability of the CRI algorithm to accurately distinguish individuals with varying tolerances to reduced central blood volume can be attributed to a unique function of the algorithm, which analyzes and compares the entirety of each waveform in a window of time to trend subtle features that correspond with varying degrees of central volume loss. This analytical advantage is based on the relationship described by the arterial waveform (ejection wave) and peripheral vascular resistance (reflected wave). As such, all mechanisms associated with compensation for central volume loss are represented in each waveform. Thus, subtle changes in waveform features, which are detected by the CRI algorithm, allow it to differentiate *individual patients* (i.e. those with high or low tolerance to central volume loss) within the first thirty beats of monitoring and every beat thereafter (29-31). The CRI algorithm estimates the following quantity:

$$CRI = 1- [BLV/BLV_{HDD}]$$

Where BLV is the current blood loss volume of the patient and BLV_{HDD} is the blood loss volume at which the patient will experience hemodynamic decompensation (SBP < 80 mm Hg) (31). CRI ranges from '1' to '0' and corresponds to the body's ability to compensate for acute intravascular volume loss (Figure 1 below). A CRI of '1' equates to supine normovolemia, while a CRI of '0' equates to being volume depleted and unable to compensate (cardiovascular collapse, SBP < 80 mm Hg). CRI values between '1' and '0' indicate the proportion of reserve remaining before hemodynamic decompensation—much like the fuel gauge of a car indicates the amount of fuel remaining in the tank. When a patient loses intravascular volume due to bleeding or dehydration, the "tank" empties and CRI goes down. With appropriate fluid resuscitation, the "tank" refills and CRI goes up. The CRI can also be affected by autonomic vascular tone. Dr. Moulton and his colleagues have subsequently shown that changes in CRI can be used to detect low volume blood loss with greater sensitivity and specificity than traditional vital signs (62,63).

CRI can also detect vascular changes associated with postural orthostatic tachycardia syndrome. Stewart et al. (64) described a patient with POTS whose traditional vital signs and CRI values were measured during positional changes. "The CRI was measured during a stand-to-supine demonstration conducted on a 16-year-old girl, who had developed postural orthostatic tachycardia syndrome (POTS) 6 years earlier. Figure [1] shows her continuously recorded HR (upper panel) and CRI values (lower panel). She demonstrated a typical tachycardic response (average HR, 120-125 bpm) when she was asked to quietly stand; her average CRI was 0.17. At 3 minutes, the subject was instructed to assume a supine posture. Within 30 seconds, the subject became clinically "normal," with a HR of 60 bpm and CRI >0.8. After 2.5 minutes in the supine posture, the subject stood up, resulting in immediate tachycardia and a drop in her CRI to nearly 0.2" (64, p. 9-10)."

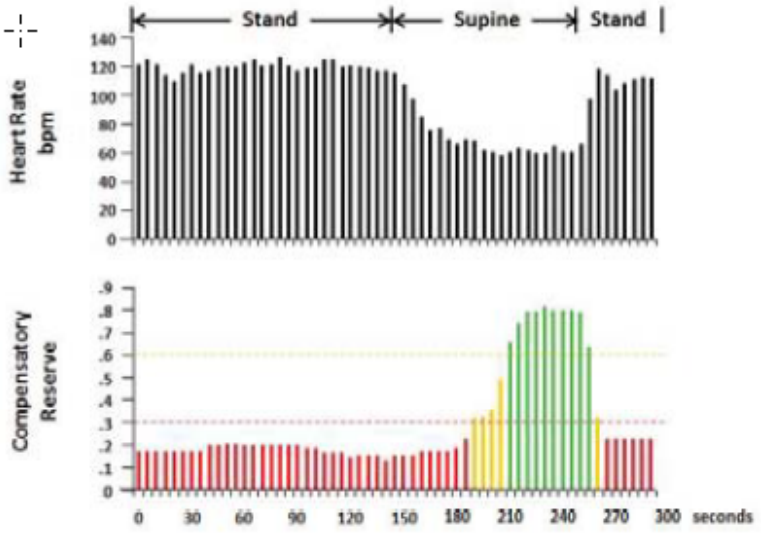

Figure 1. Heart rate (top) and compensatory reserve (bottom) measured during a 5-minute orthostatic test in a patient with postural orthostatic tachycardia syndrome (copied from 64).

Compared to traditional vital signs, CRI may be less influenced by anxiety than heart rate and may be more useful than heart rate in trending values over time. Because of the strong association between anxiety and POTS, we hypothesize that CRI may be a more accurate measure for diagnosing POTS than just relying on changes in heart rate. Moreover, CRI could greatly improve our ability to guide treatment among youth with POTS, by providing a continuous, non-invasive, physiologic parameter that is specific, sensitive, and instantaneous. Other than the one case report cited above, there are no prior studies that use CRI to evaluate POTS patients and assess if there is an association between CRI values and reported mood and functional status of the patient. Li et al. (5) utilized the FinometerPro to evaluate the hemodynamic status (with noninvasive blood

pressure monitoring) of patients with POTS, but did not have a comparison control population or an association with common symptoms. Clearly, further research is needed to determine the utility of CRI in: 1) refining the diagnostic criteria for POTS; 2) stratifying pediatric POTS patients by autonomic instability, 3) evaluating the effectiveness of various interventions; and 4) determining prognosis among youth with POTS.

Many factors can influence the validity of CRI, including vasoactive medications and conditions such as dehydration (67). This study will be executed in an outpatient setting, where there is minimal risk of the effect of vasoactive medications. In the POTS patient population, dehydration is common, with a decreased blood volume in up to 70% of patients (68, 72). While dehydration can trigger POTS symptoms, the specific etiology for the high rate of dehydration in POTS patients is poorly understood. Dehydration can also exacerbate present POTS symptoms. As a result, POTS patients are recommended to drink 2-3 L of fluids daily and supplement their salt intake for a goal of 10-12 g/day (72). Dehydration leads to decreased central blood volume and has been shown to be associated with lower CRI values in a prior study by Gagnon et al. (67). Urine specific gravity, the measure of the weight of the particles in urine, has been widely used in human studies via refractometers for assessment of fluid status (70). To better evaluate the CRI changes attributed to POTS, the hydration status of study subjects will be evaluated with urine specific gravity, and subjects with elevated urine specific gravity will be asked to drink an electrolyte-rich solution to improve their hydration status. Then, the CRI values and vital signs will be measured again in order to more accurately assess POTS patients (while they are hydrated).

Psychosocial Correlates. There is some evidence that patients with POTS are more likely to experience psychiatric problems, including depression, anxiety, and pain catastrophizing (8). These patients are sometimes misdiagnosed with a psychiatric disorder prior to an official POTS diagnosis (8). However, because studies showing a link between anxiety, depression, and pain catastrophizing in POTS have been limited by small sample sizes (e.g., n = 33 in 14) and the absence of an appropriate comparison group (e.g., 14; 15), the associations between psychiatric symptoms, functioning, and the severity of POTS are poorly understood (14, 15). Co-morbid conditions such as hypermobility may also affect the severity of POTS' symptoms. Patients with hypermobility appear to have more associated psychological issues, especially anxiety, than non-hypermobile POTS patients, even though their orthostatic measurements appear similar. In a study by Miglis et al., POTS patients with hypermobility were more likely to have outpatient visits and chronic pain symptoms (65). Further research is necessary to evaluate the differences in psychologic functioning between autonomic dysfunction patients with and without hypermobility

Additionally, sleep disorders are frequently reported in the literature on pediatric POTS, although sleep studies in adults have not shown a consistent link (7). Importantly, social-interaction factors, including social connectedness and parent-child relationships, appear to impact and be influenced by POTS symptoms; for instance, many youth with POTS express feeling socially isolated. Parents often describe difficulty in knowing "how much to push" their children to re-engage with activities, and parents express feeling stressed themselves (e.g., based on authors' clinical experiences). Research with pediatric chronic pain patients suggests that parental behaviors (e.g., accommodating, minimizing, and empathic support) and family functioning (e.g., family communication, problem solving, and satisfaction) are associated with children's functioning and pain-related disability (16, 17). Parental behaviors and family functioning are likely to play a significant role in understanding the risk for functional impairment in other chronic pediatric conditions, namely in POTS (17). However, research has not yet explored these variables among pediatric patients with POTS. Taken together, further research is very much needed to better understand to what extent psychiatric symptoms, sleep, and social interaction factors are associated with POTS. Thus, the third aim of the present study is to examine these psychosocial factors in a representative group of pediatric patients with POTS compared to healthy control subjects, in order to broaden our understanding of factors associated with POTS.

III. **Preliminary Studies/Progress Report:**

Preliminary Studies. In preparation for the current study, preliminary data have been gathered on 12 POTS patients from the CHCO Adolescent Medicine Clinic. Vital signs and CRI values were first measured in a supine position every minute for a total of 5 minutes (Phase 1). Then, the patients were asked to assume a standing position for the same measurements for 10 minutes (Phase 2). The remainder of measurements were taken when the patient resumed a supine position for 5 minutes (Phase 3). These patients were found to have an increased heart rate in a standing position, as expected, in addition to a significant drop in CRI ≥ 0.5 from their baseline supine values. Means and standard deviations for these pilot data are shown in Table 1 below; note, the method of calculating the changes in HR and CRI was based on that used in Singer et al. (2012), i.e., to compare the value 30 seconds before tilt (most closely approximated by our minute 5 of Phase 1) with the value 5 minutes into the tilt (our minute 5 of Phase 2). These preliminary results support the potential usefulness of a continuous, real-time measure of compensatory reserve as a sensitive and specific assessment of orthostatic instability. Further research is necessary to expand the size of the study group with POTS and establish a control group for comparison for vital signs and CRI values.

Table 1. Pilot Data on Heart Rate and CRI in 12 Adolescent Patients with POTS

	Heart Rate (beats per minute) *M (standard deviation)*	Compensatory Reserve Index *M (standard deviation)*
Supine (minute 5 Phase 1)	75.4 (15.7)	0.77 (0.17)
Standing (minute 5 of Phase 2)	105.6 (20.5)	0.27 (0.15)
Absolute change with standing	30.2 (12.9)	0.50 (0.17)

CRI = Compensatory Reserve Index; Phase 1 refers to first supine period, and Phase 2 refers to standing period.

Our pilot participants' heart rate increment with standing is relatively consistent with the average increment reported in the literature in adolescents with POTS (i.e., Singer et al., 2012, reported a mean of 32 bpm and standard deviation of 14 in their sample of 654 adolescent patients). This increment has been found to be significantly larger among POTS patients than healthy adolescents tested with the same procedure (e.g., mean of 27 bpm and standard deviation of 13 reported by Singer et al., 2012 in their sample of 106 normal control subjects). There are no known data on how CRI changes in response to postural changes in healthy adolescents, and thus this will be an important contribution of the present study.

Moreover, preliminary data on psychosocial factors associated with POTS have been obtained in a previous study by Dr. Pitula (with her colleague, Dr. Jessica Malmberg). By examining more than 20 adolescents with POTS as part of an intervention study, Dr. Pitula has observed that psychological factors including symptoms of anxiety and depression, social connectedness, and parental behaviors appear to be involved in predicting functional impairment resulting from POTS symptoms. Moreover, these factors seem to play an important role in predicting engagement in interventions.

Research Personnel. Members of the research team have extensive clinical and research experience in the fields of adolescent medicine and psychology generally as well as specific expertise in child and adolescent autonomic dysfunction and CRI calculation and measurement. Moreover, they have access to adolescents (12-21 years old) with autonomic dysfunction/POTS through their own practices in Adolescent Medicine as well as professional relationships with physicians in several specialty departments where patients with POTS tend to present, including cardiology, neurology, genetics, psychiatry, and gastroenterology.

Principal Investigator (PI): **Clio Pitula PhD** is a Licensed Psychologist and an Assistant Professor in the Departments of Pediatrics and Psychiatry at the University of Colorado School of Medicine (UCSOM). In her work to date, she has helped to develop and facilitate a psychological group intervention program for adolescents (13 to 18 years) with autonomic dysfunction at CHCO, as part of a research study on which she was the co-investigator. She is currently involved in a multi-disciplinary clinic within Adolescent Medicine aiming to provide assessment and brief intervention to youth with autonomic dysfunction and POTS, as well as chronic fatigue. Dr. Pitula has published multiple research studies pertaining to the functional adaptation of youth who are at-risk for mental health problems, and as such has demonstrated her high level of competence in the development, completion, analysis, and dissemination of scientific research.

Co-investigators:

Steven Moulton MD: Dr. Steven Moulton is a tenured Professor of Surgery at the University of Colorado School of Medicine and Director of Pediatric Trauma and Burn Programs at Children's Hospital Colorado. His research spans the fields of trauma and burn care, resuscitation, and hemodynamic monitoring. He co-founded Flashback technologies with Jane Mulligan and Greg Grudic, and together they developed the first, FDA cleared medical monitor (the CipherOx CRI M1) for continuous, beat-to-beat non-invasive assessment of acute blood loss. He is actively involved in research in trauma and pediatric surgery and has authored (and co-authored) more than 70 peer-reviewed articles and 15 book chapters.

David Kaplan MD: Dr. David Kaplan is a tenured Professor of Pediatrics at the University of Colorado School of Medicine, Head of Adolescent Medicine, and Chief Medical Information Officer at Children's Hospital Colorado. Throughout his career, he has concentrated on improving access to health services for adolescents by developing programs that impact the major morbidity and mortality among adolescents and young adults. His clinical practice is focused on seeing adolescents with combined medical and emotional problems, particularly patients with chronic fatigue, POTS, depression, and anxiety.

Niti Shahi MD (Primary contact): Dr. Niti Shahi is a general surgery resident from the University of Massachusetts and a current pediatric surgery research fellow at Children's Hospital Colorado (2018-2020) with a focus in trauma and burn with an interest in clinical outcomes research and machine learning with CRI. She is working on developing multiple clinical research studies including prospective and retrospective studies. She has experience in study design and data collection, leading to the publication of multiple manuscripts.

Gabrielle Shirek BA: Gabrielle is a pediatric surgery research coordinator in Dr. Moulton's Lab. She is working on multiple projects in trauma in burn and will play a key role in enrollment of study patients.

Ryan Phillips MD: Dr. Ryan Phillips is a General Surgery Resident who spending two years as a Pediatric Surgery Research Fellow at Children's Hospital Colorado. His research focuses on numerous aspects of pediatric trauma, burns, and fetal diagnosis and treatment. He is leading numerous multi-center trials where Children's Colorado is the primary study site.

David Leopold MD: Dr. David Leopold is a current Pediatric Surgery research fellow (2016-present) who has led multiple studies in the implementation of the CRI device in different patient populations. He has extensive familiarity with the machine learning based Compensatory Reserve Index and has conducted multiple prospective and retrospective investigational studies using this technology to predict hemodynamic instability and clinical outcomes.

Jamie Shoop MA: Jamie Shoop is a pre-doctoral psychology intern at Children's Hospital Colorado. Her research primarily explores social influences on risk and resilience during adolescence. Having

worked on multiple research projects, she has experience with data collection and analysis and has published multiple peer-reviewed manuscripts.

Alexandra Schwartz BA: Alexandra Schwartz is a Master of Public Health in Applied Biostatistics student at the Colorado School of Public Health. Her research experience has focused on adolescent depression and anxiety. She has worked on multiple research projects, she has experience with study design, data collection and analysis.

Other study personnel include: Rachel Workman MD, Noelle Whitney, Adam Goldsmith

IV. Research Methods

A. Description of Population to be Enrolled:

This study aims to enroll pediatric participants and their parent/legal guardian(s).

Inclusion criteria:
Adolescent participants must be aged 12 to 21 years (inclusive). Each participant must have at one parent/legal guardian who can provide informed consent for the adolescent and participate in the study by completing study questionnaires. Adolescents will be recruited into one of two groups: POTS participants and healthy controls. In order to recruit two groups of similar participants who differ primarily on whether they have POTS, healthy controls will be loosely matched to POTS participants on the basis of age, gender and race/ethnicity (i.e., Caucasian, Black or African-American, Hispanic/Latino, and other). Thus, as part of recruitment and scheduling, participants will be called for pre-screening for demographic information (i.e., age, gender, and race/ethnicity), and we will attempt to loosely approximate the demographics of POTS patients (e.g., aiming for gender and race proportions within 5-10% of POTS patients, aiming for mean age within 1-2 years). Moreover, as the majority of study measures are only validated for use in English, only English-speaking patients will be included in this study.

As discussed in the data analysis plan below, we aim to have 105 completed adolescent participants per group, i.e., 210 adolescents, plus one parent per adolescent, resulting in a total number of participating individuals of 420. As described in the application form, we will aim to recruit up to 250 adolescents to allow for drop-out during the study visit.

POTS Participants. Participants must have been diagnosed by a healthcare provider with POTS.
Healthy Controls: Participants do not have a diagnosis of any form of autonomic dysfunction.
"POTS" participants that do not meet physiologic criteria: There may be some subjects with an existing diagnosis of POTS who do not meet the physiologic criteria, i.e., an increase of heart rate greater than 40 bpm with standing. These participants will be included in the POTS group consistent with an intent to treat model. However, exploratory analyses will consider how this difference may impact study findings (e.g., primary analyses conducted with and without these participants' data; comparison of these participants to the remaining POTS participants and healthy controls).

Exclusion criteria:
Adolescents will be excluded from the study if they are outside the age range of 12-21 years old, or are wards of the state, incarcerated, decisionally impaired (i.e., have difficulty understanding the protocol during the consent process, and/or research team member is unsure of individual's ability to correctly identify whether the research study would need to stop or not), object at any time to participating in the study, have a pacemaker, are taking beta blocker medications, or were hospitalized in the past month for serious medical conditions affecting their cardiopulmonary system (note, this does not include recent hospitalizations for trauma or burns). Adolescents will also be excluded if their parent/legal guardian(s) are unwilling to participate in the study and/or do not have legal custody of the adolescent participant. Lastly, adolescents who do not speak English will be excluded from the study as majority of the study measures are only validated in English.

B. Outcome Measures:

For adolescent participants:

1. Demographic information (from pre-screening phone call and intake form): Gender, race, ethnicity, Date of screening. Parents will also be asked about their household income and highest education level.
2. **Measurement of CRI values** and changes in CRI will be conducted with a supine position for 5 minutes, followed by a standing position for 10 minutes, and then a return to supine position for 5 minutes (see below for rationale). The time on the device (and device number) will be recorded prior to CRI value collection in addition to the actual time from a room clock or wristwatch. The time of CRI measurement (and thus the entire study visit) will be standardized to afternoon.
3. **Measurement of Orthostatic Vital Signs**: Traditional orthostatic vital signs will be measured concurrently with CRI values. In POTS patients, after a supine position, heart rate is expected to increase >40 bpm (beats per minute) within *10 minutes* of an upright position (78,79). We plan to conduct measurements with an initial supine position for 5 minutes, standing position for 10 minutes, and then a supine position for 5 minutes for both measures. Height and weight will be obtained by research staff.
4. **Urine specific gravity will be measured and reported for each patient via the use of a refractometer.**
5. **Hypermobility**: The Beighton Score is a validated measure used to evaluate hypermobility in children on a scale of 0-9 for different joints using 3 score bands (32).
6. **History intake form:** A brief history will be conducted by research staff (jointly with the adolescent participant and his/her parent). The following measures will be obtained:
    a) **Onset of symptoms (month and year)**
    b) **Diagnosis of POTS (month and year)**
    c) **List of medications and dosages**
    d) **Current treatment regimen:** We will ask parents and subjects about their current treatment regimen for POTS including medications, salt supplementation, and current therapy (e.g., physical therapy, psychiatry, psychology).
    e) **Other medical diagnoses.**
7. **Survey utilization:**
    1. **List of physical symptoms:** Patients will be asked about the frequency of physical symptoms on a scale of 0-3 (Not at all-0, Several days-1, More than half the days-2, Nearly every day-3) for the following symptoms: Fatigue, dizziness/syncope, weakness, headaches, chest pain, heart racing/palpitations, sweating, shortness of breath/diaphoresis, abdominal (tummy) pain, nausea, vomiting, menses (periods) pain, cry easily, altered temperature sensation/heat intolerance, discoloration of extremities, peripheral edema/venous pooling, numbness, brain fog, diarrhea, constipation, blurred vision, muscle pain, joint pain, exercise intolerance/shortness of breath with activity, insomnia/trouble sleeping, joint hypermobility, sensitivity to light/photophobia, early satiety, and other (11, 33). Each symptom endorsed will be multiplied by its frequency (i.e., 0, 1, 2, or 3) to result in a total score representing the sum of weighted items.
    2. **Anxiety/Stress frequency:** This scale asks patients to rate their perception of the frequency of anxiety and stress experienced from environmental influences on a scale of 0-3 (Not at all-0, Several days-1, More than half the days-2, Nearly every day-3).
    3. **Sleep survey:** Insomnia subscale and one sleep quality item drawn from Children's Report of Sleep Patterns, total 7 items, Meltzer et al. and consistent with the Pittsburgh Sleep Quality index described by Buysse (34, 35).
    4. **Revised Child Anxiety and Depression Scale (RCADS and RCADS-P):** The RCADS is a well-established survey tool used to assess symptoms of anxiety and depression, and this tool has been validated in cross-cultural samples with over 88,000 children (36).

There is also evidence that this measure retains its validity when used in adult samples (i.e., 18 plus) (76).

5. **Functional disability inventory (FDI):** The Functional disability inventory, established by Walker and Greene, is a valid measure of perceived activity limitations in children and adults (39). Participants will be asked to complete a 15-question survey to evaluate the difficulty of daily tasks with a scale of impossible (4), a lot of trouble (3), some trouble (2), a little trouble (1), and no trouble (0) (39). This measure will be adapted very slightly to reflect the ubiquity of electronic media for today's adolescents, i.e., question 11 will be modified from "Watching TV" to "Watching TV and other screens." This measure yields an overall summed score.
6. **Social Connectedness Scale:** The Social Connectedness Scale, developed by Lee, assesses the responder's perceptions of social connectedness (i.e., the perception of interpersonal closeness with the social world) through 15 self-report items (please refer to Lee, Dean, & Jung, 2008, for 15-item version compared with 20 item version) (40, 41). Note, for adolescents in college, we will administer the Campus Connectedness Scale, which is an adaptation of the SCS by the same authors.
7. **Friendship:** The "Close Friend Support/Regard" subscale of the Social Support Scale for Children and Adolescents (42, 43) will be used to assess the adolescent's perception that he/she has a close friend who he/she can tell problems to, who truly understands him/her, who he/she can complain to about things that bother them, who he/she can spend time with, and who really listens to what he/she says. This subscale comprises 6 items rated on a Likert scale from "really true for me" and "sort of true for me" for the positive phrasing of the item (e.g., "Some teenagers have a close friend who really understands them") to "sort of true for me" and "really true for me" for the negative phrasing of the item (e.g., "Other teenagers don't have a close friend who really understands them."). Given that some participants will be above 18 years, we will modify the wording to "some people".
8. **Frequency of friendship contact:** Participants will be asked to indicate, over the past month, on how many occasions they have spent time with a close friend outside of school (i.e., free entry).
9. **Family Assessment Device General Functioning scale (GFS):** The Family Assessment GFS is a twelve-point scale that evaluates the "overall health or pathology in family functioning" via analysis of six dimensions of family functioning: problem solving, communication, roles, affective responsiveness, affective involvement, and behavior control (44-46). This scale has been validated with established psychometrics in multiple prior studies with comparison to other self-reported family measurements (44, 47).
10. **Sexual maturity Rating (Tanner stage)**: This questionnaire with image demonstrations of the different Tanner stages will ask patients to self-report their appropriate Tanner stages (55, 56). The Tanner Sexual Maturity Rating has been widely utilized for evaluation of growth and development and endorsed by the Bright Futures AAP guidelines (74). Self-assessment of Tanner stages has been validated in the adolescent population (75). In female patients, the scale will document the Tanner stages in respect to of breasts and pubic hair. In male patients, the scale will document the presence of a pubic hair. Subjects will be asked to complete a survey with images representing their own pubertal development (56).

<u>For the parent/legal guardian(s):</u>

1. **Functional disability inventory (FDI):** The Functional disability inventory, established by Walker and Greene, is a valid measure of perceived activity limitations in children and adults (39). Parents will be asked to complete a 15-question survey to evaluate the difficulty of daily tasks with a scale of impossible, a lot of trouble, some trouble, a little trouble, and no trouble (39). This measure will be adapted very slightly to reflect the ubiquity of electronic media for today's adolescents, i.e., question 11 will be modified from "Watching TV" to "Watching TV and other screens."

2. **Revised Child Anxiety and Depression Scale – Parent Version (RCADS-P):** The Revised Child Anxiety and Depression Scales-Parent Version (RCADS-P) is a 47-question survey created for parents to assess parents' perception of symptoms of anxiety and depression in their children. (37, 38).
3. **Family Assessment Device General Functioning scale (GFS):** The Family Assessment GFS is a twelve-point scale that evaluates the "overall health or pathology in family functioning" via analysis of six dimensions of family functioning: problem solving, communication, roles, affective responsiveness, affective involvement, and behavior control (44-46). This scale has been validated with established psychometrics in multiple prior studies with comparison to other self-reported family measurements (44, 47). A score greater than 2 for each question was determined as the cut-off for a healthy response (47). These instruments will be framed in light of living with their child with POTS symptoms, to reduce the likelihood that parents will feel blamed due to being asked these questions, i.e., "*There are many ways in which caring for a young person with chronic illness can affect people's lives. Below are some statements that may or may not apply to you."*
4. **Parent Behavior Adult Responses to Children's Symptoms** (**ARCS):** The ARCS is a 29-point scale with a goal of assessment of parent responses to their children's symptoms (i.e. protectiveness, minimization, encouragement, and monitoring)**.** Existing research suggests that parental response style is associated with functional impairment and mental health in chronic pain populations (16). Less is known about the role that parental response style plays in understanding physical and emotional functional in pediatric POTS patients, despite calls from researchers to explore this issue (48). The psychometrics of the ARCS has been studied in regard to the specific factors: protect, minimize, and encourage and monitor (49).
5. **Parent Mental Health (PHQ-9, GAD-7):** Parents of children with chronic medical conditions tend to experience higher levels of anxious and depressive symptoms (50). The chronic pain literature suggests that parent mental health and child functioning are closely linked, such that parents who are functioning better tend to have children who are functioning better, as well (51). Additional research from the chronic pain literature suggests that parent mental health may directly impact parental response to children's symptoms (52). The Parent Mental Health scales, Parent Health Questionnaire 9 item scale (PHQ-9) and Generalized Anxiety Disorder 7 item scale (GAD-7), are short self-reported questionnaires that are widely used with established psychometrics in the parent population (53, 54).

C. Study Design and Research Methods

A control group of 100 participants and a group of 100 participants with POTS will be recruited for the study. The subjects and their parents will participate in one research clinic visit for the study.

Recruitment of POTS participants:

Participants with POTS 12 to $\leq$21 years old will be recruited from clinics in Children's Hospital Colorado including those in the departments of Adolescent Medicine, Neurology, Gastroenterology, Cardiology, and Genetics. To determine the pediatric POTS population, a search of medical records by ICD codes in EPIC for POTS using the Snomed grouper for Postural orthostatic tachycardia syndrome (Concept ID C1299624, includes ICD 10 code I49.8 and ICD 9 code 337.9) will be conducted. Research team members will first identify potential subjects through their clinics with whom they have a treatment relationship (e.g., Dr, Kaplan's Adolescent Medicine clinic). A letter briefly describing the study will be sent to these patients (and/or their parents) to inform them about the study and provide them with the research team's contact information. For patients who do not have a treatment relationship with research team members, we will provide the patient's provider with this letter template; they will also be provided with a HIPAA-A form to allow us to contact families they see in their clinics, and/or they can give interested families our research team's contact information. Additional advertisement for the study will be provided through posters placed in CHCO

clinics; emails will be sent to providers identified as working with these patients, and an advertisement will be placed on the University of Colorado Clinical Trials website. Subjects will be financially compensated $50.00 for participating in the study, and they will be provided with a letter from the study team and a certificate acknowledging their contributions to the research study. Once study patients have been recruited for the study, the patient and their parent(s) will be called to set up a research appointment to complete the evaluation of their CRI and psychosocial assessments. If more than one parent comes to the clinic appointment, they will both be offered the opportunity to complete the surveys. These surveys will be built into REDcap and completed by parents and their child on an iPad.

Recruitment of Healthy Controls:
Healthy pediatric subjects 12 to $\leq$21 years old will be recruited under the clinical relationship they have with research team members (e.g., Dr, Kaplan's Adolescent Medicine clinic). We will also use poster advertisements, UC Clinical Trials website, and email local pediatricians in private practice in the Denver/Aurora area to recruit control patients, which will likely be a better demographic match for the POTS patients. We will request the patient's providers fill out a HIPAA-A form and ask that the provider give the families our contact information as well. Financial compensation of $50.00 will be offered for involvement in the study, and participants will be provided with a letter from the study team and a certificate acknowledging their contributions to the research study. Once study patients have been recruited for the study, the patient and their parent(s) will be called to set up a research appointment for evaluation of their CRI and psychologic assessments. If more than one parent comes to the clinic appointment, they will both be offered the opportunity to complete the surveys. These surveys will be built into REDcap and emailed to parent(s) and their child for remote completion.

Consent process:
If agreeable to participation, potential subjects and their parent/legal guardian(s) will go through the consent process. A COMIRB approved consent form or assent form will be used as appropriate based on the potential subject's age. A signed and dated copy of the consent form will be provided to all subjects via email. Consent/assent will be obtained by a meeting with the potential subject (and their parent(s) if the potential subject is a child) and a member of the research team over the phone using an eConsent form available through RedCap. The link to the consent form will be sent to potential participants for review before and during the phone meeting. The research team member will be trained on this specific protocol, trained through required Research and HIPAA CITI courses, trained on the research consent process, and supervised by the PI or their delegate, and will be listed and approved on the COMIRB personnel form. Given the clinical relationships that some investigators will have with the potential subjects, to minimize the possibility of coercion or undue influence, medical providers involved in the patient's treatment will not be consenting potential subjects. Other research personnel not involved in the patient's treatment will do the consent process with the potential subjects and will make it clear that this is for research, that their participation is voluntary, and that they will receive the same medical care regardless of if they participate in the research study. The consent process will happen in a quiet and private setting over the phone with the use of an eConsent accessible via RedCap. Potential subjects will be given time to read the form, ask questions, and have time to consider whether or not to be involved in this study. The research personnel obtaining consent will ask questions to verify that the potential subject has understood the consent or assent form. The research personnel will also follow a checklist to make sure that all required parts of the consent process have happened appropriately. If blind, illiterate, or those with similar reading limitations desire to participate in the study, the entire consent form will be read to the potential subjects and a member of the clinical staff or a member of the Adolescent Medicine Clinic staff (who are not on the research team) will serve as a witness.

Parents of participants 18 or older will be allowed to complete the surveys remotely. A postcard consent will be used for this process, and the participant will be consented in person in the clinic.

Study clinic appointment:

The participant and accompanying parent(s) will be met by a member of the research team after arriving to the research space. The informed consent/assent process will then be completed by member of the research team. The participant will then be met by a nursing staff member or co-investigator for urine specific gravity and measurement of CRI and other vitals (approx. 30 mins). In the literature, there are varying numerical cutoffs for normal hydration ranging from 1.006 to 1.029, and most of these studies have been conducted in adults (84). However, one of the most widely accepted values for normal hydration from urine specific gravity was established by the American College of Sports Medicine; they released a statement with the determination of a urine specific gravity $\leq$1.020 "is indicative of being euhydrated (83, 84)," (note, euhydrated refers to normal hydration). Thus, if urine specific gravity is too high (>1.020) as measured via spectrometer, the participant will be asked to drink 1-2 8 oz bottles of electrolyte-rich solution (i.e., Gatorade) and return for CRI and other vitals measurement after approximately 1-2 hours. In the meantime, patients will be asked to complete the surveys while waiting for rehydration. After they complete the surveys, the urine specific gravity will be re-checked, and if it is <1.020, then CRI measurements will be checked. To maximize likelihood that participants will be sufficiently hydrated, they will be encouraged to drink fluids prior to the appointment start (i.e., when confirming the appointment by phone and when arriving to the research facility). All research staff will be trained on collecting and measuring urine specific gravity with a refractometer with samples with different specific gravity values. The hand-held refractometer is an optical instrument manufactured by Kibeland that will be utilized by all the research staff. It uses a few drops of solution for measurement of the specific gravity, and it has automatic temperature compensation.

After these measures are complete (or while waiting for hydration), a research team member will instruct the participant to complete surveys on electronic tablet and will administer some interview items. The parent will also be instructed to complete his/her surveys and interview items during this time. Once surveys, interview items, and vitals are complete, the participant and parent(s) will be paid by a member of the research team and excused.

CRI measurement during study clinic appointment:
A FDA cleared and/or investigational CipherOx CRI M1 devices will be used for CRI data collection. The devices are equivalent and run the same exact software package; the only difference is that the FDA cleared devices are new and manufactured by an ISO compliant manufacturer (Evergreen Technologies). These devices use a standard pulse oximetry sensor, which will be attached to the CRI device and the participant's right or left index finger. The participant will lie down on an examination table for 5 minutes, then will stand for 10 minutes, then return to the supine position for 5 minutes (all while the pulse oximeter is obtaining continuous CRI readings).

V. Description, Risks and Justification of Procedures and Data Collection Tools:

Risks to the subject are minimal. Non-invasive physiological waveform data will be collected using non-invasive sensors. The research for this study does not involve specialized procedures or changes to the standard of care for patients enrolled in this study; therefore, there are minimal risks to patients who will be enrolled in this study. Flashback Technologies' FDA cleared CipherOx CRI M1 devices will be used for data collection, however, CRI will not be displayed or available to the clinicians during the data collection process.

Risks to the subject associated with continuous, non-invasive blood pressure measurements from finger cuffs are minimal and include:

- slight discomfort from the cuffs inflating around a finger
- water blisters may occur under the cuffs (this is rare—it has only happened to one subject to our knowledge)
- during measurement, some coloring of the fingertip may occur; the coloring will disappear within a few minutes after removing the cuff

Risks to the subject associated with pulse oximetry are minimal and include:

- Slight discomfort from applying the finger sensor to the subject's finger
- Temporary pressure marks on the finger resulting from application of the sensor

The risk of syncope is no greater than in routine clinical visits or getting out of bed after sleeping at night. If the patient feels dizzy, we will have them resume a supine position until the dizziness resolves.

All devices and equipment used for monitoring and collecting data must pass the appropriate hospital Biomedical Engineering electrical safety testing. There are very minimal risks associated with filling out questionnaires (which will be directly uploaded to REDcap) in the outpatient clinic, and this study does not affect patient care. The study is voluntary. There is a potential risk of patient information being accidentally seen by someone who is not on the research protocol. The risk is minimal, and usually not serious. All protected information that is accessed and obtained as part of this protocol will be kept on a Children's Hospital Colorado encrypted computer and server. Computer and server access at Children's is protected with a password. The computers are kept in a badge access only area of the hospital (the administrative pavilion). Furthermore, protected health information will be kept separate from other research information. Once data analysis is complete, all data related to individual protected health information will be destroyed.

Participants and their parents might feel uncomfortable or upset when completing study measures and answering personal questions about themselves and their children. This risk is minimal. Risk will be mitigated by providing explanation during informed consent that participants have the right to refrain from completing study measures, and to skip questions that are difficult and/or make them uncomfortable. Participants will also be informed that their participation is voluntary so that they can withdraw from the study at any time, and that their participation/withdrawal from the study does not influence their current and/or future ability to access therapeutic services. A licensed psychologist (Dr. Pitula) will also be available to debrief families over the phone if needed.

VI. Data Safety Monitoring Plan: Data will be managed in a secure and HIPAA-compliant manner. The data that is collected by Flashback Technologies' pulse oximeters is collected in a de-identified fashion and stored on a drive internal to the device itself. These devices will be kept in locked rooms or locked carts within areas of the campus buildings that require badges to enter. Identifiable data will be kept on computers that are password protected and can only be accessed by members of the research team. These computers are also kept in areas of campus buildings which require badges to enter. De-identification of data will occur after the waveform data is collated with pertinent medical record data. The paper informed consent forms and CRI data collection sheets will be stored in the locked filing cabinets at the desk of a member of the research team (which is also inside an area of campus buildings which require badges to enter). The surveys will be directly conducted in REDCap. The CRI data (from data collection sheets) will be manually transcribed into REDCap as well. The CRI data from the device (uploaded to the computer) will also be uploaded into REDCap.

If the surveys uncover suicidality in the subjects (adolescents and/or parents), this will be addressed by the psychologist, who will conduct a risk assessment, and a report will be made if necessary (e.g., calling 911 or accompanying the participant to the emergency department).

VII. Potential Scientific Problems:

The physiological responses of our populations of interest may be different from those on which the CRI algorithm was built. While we have no reason to believe otherwise, there is no way to be certain if the CRI algorithm will be effective in tracking the primary outcomes measures of our study. Since

this is a survey study, there is a risk of recall bias. Given the length of the survey, there is a risk of survey fatigue for subjects and incomplete completion of the survey and missing information.

IX. Data Analysis Plan:

All data will be stored and managed in REDCap. Subjects (both children and parents) will be asked to complete the surveys on an iPad in clinic. Data analysis plan is outlined for each study aim below.

Aim #1: To examine group differences in CRI, the primary outcome variable will be the absolute change in CRI from supine (i.e., supine minute 5) to standing (i.e., standing minute 5). Use of this primary outcome variable is modeled after the primary outcome variable used by Singer et al., 2012 (i.e., HR increment from baseline at 5 minutes post-tilt); note, secondary outcome variables used by Singer and colleagues included values at baseline and 1, 5, and 10 minutes post-tilt, as well as change from baseline at 1 minute and 10 minutes post-tilt. Linear regression analysis will be used to assess the influence of age and BMI on variables. The chi-squared test will be used to assess for the influence of sex and Tanner stage. Because previous research suggests that these data may be non-parametric, Mann-Whitney Wilcoxon testing will be used to assess for differences in variables between patients and controls. Significance will be set at the 5% level.

As this is the primary study aim, power analysis was conducted to assess sample size needed to adequately fulfill this aim. Power analysis was conducted using the program *G*Power* (57) to determine the sample size needed to detect a true difference in CRI decrement between patient and control groups given likely effect size (i.e., 0.36, based on group means for heart rate increment reported by Singer et al., 2012) employing the traditional statistical analysis criteria of 5% alpha error probability and 80% power (58). Given these parameters, power analysis indicated that a sample size of 103 participants per group would be necessary to detect a true difference in CRI decrement between groups. As a result, we aim to have 105 participants per group in order to be adequately powered to detect a group difference in our primary outcome variable.

Aim #2: To assess associations between CRI values, symptoms of POTS, and functional impairment, regression analyses will be used. Thus, in hierarchical linear regression, two separate regression analyses would be conducted with POTS symptom score (i.e., continuous variable representing the sum of weighted items) and functional impairment score (i.e., sum of FDI total score for parent and child report) as outcomes. In each analysis, covariates (e.g., age, sex) would be entered in the first step and CRI value (i.e., two sets of analyses using a) CRI decrement from supine to standing, as defined above, and b) lowest CRI value attained when standing) entered in the second step. Analyses would be corrected for multiple comparisons using Boneferroni correction. Exploratory analyses will also consider how comorbid hypermobility influences the associations between CRI values and severity and impact of POTS (e.g., by comparing youth with POTS and hypermobility to youth with POTS without hypermobility).

Aim #3: To examine differences in psychosocial factors between POTS and control groups, separate scores will be used for each psychosocial variable, i.e., symptoms of anxiety and depression (RCADS total anxiety T score, RCADS depression subscale T score, separate scores for parent and child report), sleep quality (mean of 7 items), social connectedness (mean score on SCS), friendship (mean score on Close Friend Support subscale), family functioning (mean score on GFS, separate scores for parent and child report), parent behavior (mean score on ARCS), and parent mental health (sum of GAD-7 and PHQ-9 sum scores), resulting in 11 separate continuous outcome variables. First, individual outcomes will be assessed first using independent paired sample t-tests with group as the between factor. Psychosocial variables showing no independent relationships to the groups will be discarded, and those showing a group difference will be entered into a Multiple Analysis of Variance (MANOVA). Group (i.e., POTS versus control) will be the between factor, and age, sex, and/or Tanner stage can be used as covariates if they are shown to be associated with the outcome variables in preliminary analyses.

X. Summarize Knowledge to be gained:

Measurement of CRI in children with POTS and the association with somatic and psychological symptoms is expected to help us better understand how to assess the severity of their disease and will likely inform more effective interventions for these patients.

XI. Budget: In attached spreadsheet

XII. References:

1. Lambert E, Lambert GW. Sympathetic dysfunction in vasovagal syncope and the postural orthostatic tachycardia syndrome. Frontiers in physiology. 2014;5:280.

2. Fu Q, Levine BD. Exercise in the postural orthostatic tachycardia syndrome. Autonomic Neuroscience. 2015;188:86-9.

3. Rowell LB. Human cardiovascular control. Oxford University Press, USA; 1993.

4. Jacob G, Shannon JR, Costa F, Furlan R, Biaggioni I, Mosqueda-Garcia R, et al. Abnormal norepinephrine clearance and adrenergic receptor sensitivity in idiopathic orthostatic intolerance. Circulation. 1999 Apr 6;99(13):1706-12.

5. Li H, Liao Y, Wang Y, Liu P, Sun C, Chen Y, et al. Baroreflex sensitivity predicts short-term outcome of postural tachycardia syndrome in children. PloS one. 2016;11(12):e0167525.

6. Zhao J, Han Z, Zhang X, Du S, Liu AD, Holmberg L, et al. A cross-sectional study on upright heart rate and BP changing characteristics: basic data for establishing diagnosis of postural orthostatic tachycardia syndrome and orthostatic hypertension. BMJ open. 2015;5(6):e007356.

7. Stewart JM, Boris JR, Chelimsky G, Fischer PR, Fortunato JE, Grubb BP, et al. Pediatric Disorders of Orthostatic Intolerance. Pediatrics. 2018 Jan;141(1):10.1542/peds.2017,1673. Epub 2017 Dec 8.

8. Grigoriou E, Boris JR, Dormans JP. Postural orthostatic tachycardia syndrome (POTS): association with Ehlers-Danlos syndrome and orthopaedic considerations. Clinical Orthopaedics and Related Research®. 2015;473(2):722-8.

9. Johnson JN, Mack KJ, Kuntz NL, Brands CK, Porter CJ, Fischer PR. Postural orthostatic tachycardia syndrome: a clinical review. Pediatr Neurol. 2010;42(2):77-85.

10. Kizilbash SJ, Ahrens SP, Bruce BK, Chelimsky G, Driscoll SW, Harbeck-Weber C, et al. Adolescent fatigue, POTS, and recovery: a guide for clinicians. Current problems in pediatric and adolescent health care. 2014;44(5):108-33.

11. Boris JR, Bernadzikowski T. Demographics of a large paediatric postural orthostatic tachycardia syndrome program. Cardiol Young. 2018;28(5):668-74.

12. Kritzberger CJ, Antiel RM, Wallace DP, Zacharias JD, Brands CK, Fischer PR, et al. Functional disability in adolescents with orthostatic intolerance and chronic pain. J Child Neurol. 2011;26(5):593-8.

13. Pederson CL, Brook JB. Health-related quality of life and suicide risk in postural tachycardia syndrome. Clinical autonomic research. 2017;27(2):75-81.

14. Bruce BK, Weiss KE, Harrison TE, Allman DA, Petersen MA, Luedkte CA, et al. Interdisciplinary treatment of maladaptive behaviors associated with postural orthostatic tachycardia syndrome (POTS): a case report. Journal of clinical psychology in medical settings. 2016;23(2):147-59.

15. Lewis I, Pairman J, Spickett G, Newton JL. Clinical characteristics of a novel subgroup of chronic fatigue syndrome patients with postural orthostatic tachycardia syndrome. J Intern Med. 2013;273(5):501-10.

16. Claar RL, Guite JW, Kaczynski KJ, Logan DE. Factor structure of the Adult Responses to Children's Symptoms: validation in children and adolescents with diverse chronic pain conditions. Clin J Pain. 2010;26(5):410-7.

17. Palermo TM, Putnam J, Armstrong G, Daily S. Adolescent autonomy and family functioning are associated with headache-related disability. Clin J Pain. 2007;23(5):458-65.

18. Marik PE, Cavallazzi R, Vasu T, Hirani A. Dynamic changes in arterial waveform derived variables and fluid responsiveness in mechanically ventilated patients: a systematic review of the literature. Crit Care Med. 2009 Sep;37(9):2642-7.

19. Brasel KJ, Guse C, Gentilello LM, Nirula R. Heart rate: is it truly a vital sign? J Trauma. 2007 Apr;62(4):812-7.

20. Haensch C, Tosch M, Katona I, Weis J, Isenmann S. Small-fiber neuropathy with cardiac denervation in postural tachycardia syndrome. Muscle Nerve. 2014;50(6):956-61.

21. King RW, Plewa MC, Buderer NMF, Knotts FB. Shock index as a marker for significant injury in trauma patients. Acad Emerg Med. 1996;3(11):1041-5.

22. Convertino VA, Ryan KL, Rickards CA, Salinas J, McManus JG, Cooke WH, et al. Physiological and medical monitoring for en route care of combat casualties. Journal of Trauma and Acute Care Surgery. 2008;64(4):S342-53.

23. McGrath SP, Ryan KL, Wendelken SM, Rickards CA, Convertino VA. Pulse oximeter plethysmographic waveform changes in awake, spontaneously breathing, hypovolemic volunteers. Anesthesia & Analgesia. 2011;112(2):368-74.

24. Convertino VA, Moulton SL, Grudic GZ, Rickards CA, Hinojosa-Laborde C, Gerhardt RT, et al. Use of advanced machine-learning techniques for noninvasive monitoring of hemorrhage. J Trauma. 2011 Jul;71(1 Suppl):S25-32.

25. Chan GS, Middleton PM, Lovell NH. Photoplethysmographic variability analysis in critical care—current progress and future challenges. Engineering in Medicine and Biology Society, EMBC, 2011 Annual International Conference of the IEEE; IEEE; 2011.

26. Convertino VA. Lower body negative pressure as a tool for research in aerospace physiology and military medicine. J Gravit Physiol. 2001 Dec;8(2):1-14.

27. Convertino VA, Ludwig DA, Cooke WH. Stroke volume and sympathetic responses to lower-body negative pressure reveal new insight into circulatory shock in humans. Autonomic Neuroscience. 2004;111(2):127-34.

28. Cooke WH, Rickards CA, Ryan KL, Kuusela TA, Convertino VA. Muscle sympathetic nerve activity during intense lower body negative pressure to presyncope in humans. J Physiol (Lond). 2009;587(20):4987-99.

29. Ryan KL, Batchinsky A, McManus JG, Rickards CA, Convertino VA. Changes in Pulse Character andMental Status Are Late Responses to Central Hypovolemia. Prehospital Emergency Care. 2008;12(2):192-8.

30. Convertino VA, Grudic G, Mulligan J, Moulton S. Estimation of individual-specific progression to impending cardiovascular instability using arterial waveforms. J Appl Physiol. 2013;115(8):1196-202.

31. Moulton SL, Mulligan J, Grudic GZ, Convertino VA. Running on empty? The compensatory reserve index. J Trauma Acute Care Surg. 2013 Dec;75(6):1053-9.

32. Smits-Engelsman B, Klerks M, Kirby A. Beighton score: a valid measure for generalized hypermobility in children. J Pediatr. 2011;158(1):119,123. e4.

33. Boris JR, Bernadzikowski T. Utilisation of medications to reduce symptoms in children with postural orthostatic tachycardia syndrome. Cardiol Young. 2018:1-7.

34. Meltzer LJ, Avis KT, Biggs S, Reynolds AC, Crabtree VM, Bevans KB. The Children's Report of Sleep Patterns (CRSP): a self-report measure of sleep for school-aged children. Journal of Clinical Sleep Medicine. 2013;9(03):235-45.

35. Buysse DJ, Reynolds III CF, Monk TH, Hoch CC, Yeager AL, Kupfer DJ. Quantification of subjective sleep quality in healthy elderly men and women using the Pittsburgh Sleep Quality Index (PSQI). Sleep. 1991;14(4):331-8.

36. Piqueras JA, Martín-Vivar M, Sandin B, San Luis C, Pineda D. The Revised Child Anxiety and Depression Scale: A systematic review and reliability generalization meta-analysis. J Affect Disord. 2017;218:153-69.

37. Becker SP, Schindler DN, Luebbe AM, Tamm L, Epstein JN. Psychometric Validation of the Revised Child Anxiety and Depression Scales–Parent Version (RCADS-P) in Children Evaluated for ADHD. Assessment. 2017:1073191117735886.

38. Ebesutani C, Chorpita BF, Higa-McMillan CK, Nakamura BJ, Regan J, Lynch RE. A psychometric analysis of the Revised Child Anxiety and Depression Scales—Parent version in a school sample. J Abnorm Child Psychol. 2011;39(2):173-85.

39. Walker LS, Greene JW. The functional disability inventory: measuring a neglected dimension of child health status. J Pediatr Psychol. 1991;16(1):39-58.

40. Lee RM, Robbins SB. Measuring belongingness: The social connectedness and the social assurance scales. Journal of counseling psychology. 1995;42(2):232.

41. Lee RM, Dean BL, Jung K. Social connectedness, extraversion, and subjective well-being: Testing a mediation model. Personality and Individual Differences. 2008;45(5):414-9.

42. Harter S. Social support scale for children. University of Denver, Department of psychology; 1985.

43. Harter S. Social support scale for children: Manual and questionnaires. Denver, CO: University of Denver. 2012.

44. Byles J, Byrne C, Boyle MH, Offord DR. Ontario Child Health Study: reliability and validity of the general functioning subscale of the McMaster Family Assessment Device. Fam Process. 1988;27(1):97-104.

45. Anttila P, Sourander A, Metsähonkala L, Aromaa M, Helenius H, Sillanpää M. Psychiatric symptoms in children with primary headache. Journal of the American Academy of Child & Adolescent Psychiatry. 2004;43(4):412-9.

46. Epstein NB, Baldwin LM, Bishop DS. The McMaster family assessment device. J Marital Fam Ther. 1983;9(2):171-80.

47. Miller IW, Epstein NB, Bishop DS, Keitner GI. The McMaster family assessment device: reliability and validity. J Marital Fam Ther. 1985;11(4):345-56.

48. Keating EM, Antiel RM, Weiss KE, Wallace D, Antiel SJ, Fischer PR, et al. Parental perceptions of pediatric pain and POTS-related disability. Clin Pediatr. 2017;56(13):1185-92.

49. Van Slyke DA, Walker LS. Mothers' responses to children's pain. Clin J Pain. 2006;22(4):387-91.

50. Campo JV, Bridge J, Lucas A, Savorelli S, Walker L, Di Lorenzo C, et al. Physical and emotional health of mothers of youth with functional abdominal pain. Arch Pediatr Adolesc Med. 2007;161(2):131-7.

51. Benore E, Brenner A, Banez GA, Wang L, Worley S. It takes two: Parent functioning within the pediatric chronic pain experience and interdisciplinary rehabilitation treatment. Rehabilitation psychology. 2018;63(2):205.

52. Noel M, Alberts N, Langer SL, Levy RL, Walker LS, Palermo TM. The sensitivity to change and responsiveness of the adult responses to children's symptoms in children and adolescents with chronic pain. J Pediatr Psychol. 2015;41(3):350-62.

53. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. Journal of general internal medicine. 2001;16(9):606-13.

54. Spitzer RL, Kroenke K, Williams JB, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. Arch Intern Med. 2006;166(10):1092-7.

55. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics. 1980 Dec;66(6):918-20.

56. Taylor SJ, Whincup PH, Hindmarsh PC, Lampe F, Odoki K, Cook DG. Performance of a new pubertal self-assessment questionnaire: a preliminary study. Paediatr Perinat Epidemiol. 2001 Jan;15(1):88-94.

57. Faul F, Erdfelder E, Lang A, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods. 2007;39(2):175-91.

58. Singer W, Sletten DM, Opfer-Gehrking TL, Brands CK, Fischer PR, Low PA. Postural tachycardia in children and adolescents: what is abnormal? J Pediatr. 2012;160(2):222-6.

59. Hershey AD, Powers SW, Vockell AL, LeCates S, Kabbouche MA, Maynard MK. PedMIDAS: development of a questionnaire to assess disability of migraines in children. Neurology. 2001 Dec 11;57(11):2034-9. 60. Hinojosa-Laborde C, Howard JT, Mulligan J, Grudic GZ, Convertino VA. Comparison of compensatory reserve during lower-body negative pressure and hemorrhage in

nonhuman primates. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2016;310(11):R1154-9.

61. Armed Forces Health Surveillance Center: Syncope, active and reserve components, U.S. Armed Forces, 1998-2012. Medical Surveillance Monthly Report 2013; 20(11): 5-9.

62. Stewart CL, Mulligan J, Grudic GZ, Convertino VA, Moulton SL. Detection of low-volume blood loss: compensatory reserve versus traditional vital signs. J Trauma Acute Care Surg 2014;77: 892–898.

63. Nadler R, Convertino VA, Gendler S, Lending G, Lipsky AM, Cardin S, Lowenthal A, Glassberg E. The value of noninvasive measurement of the compensatory reserve Index in monitoring and triage of patients experiencing minimal blood loss. Shock 2014;42: 93–98.

64. Stewart CL, Nawn CD, Mulligan J, Grudic G, Moulton SL, Convertino VA. Compensatory reserve for early and accurate prediction of hemodynamic compromise: Case studies for clinical utility in acute care and physical performance. J Spec Oper Med 2016;16:6-13.

65. Miglis MG, Schultz B, Muppidi S. Postural tachycardia in hypermobile Ehlers-Danlos syndrome: A distinct subtype? Autonomic Neuroscience. 2017;208:146-9.

66. Plash WB, Diedrich A, Biaggioni I, Garland EM, Paranjape SY, Black BK, et al. Diagnosing postural tachycardia syndrome: comparison of tilt testing compared with standing haemodynamics. Clin Sci (Lond). 2013 Jan;124(2):109-14.

67. Gagnon D, Schlader ZJ, Adams A, Rivas E, Mulligan J, Grudic GZ, et al. The Effect of Passive Heat Stress and Exercise-Induced Dehydration on the Compensatory Reserve During Simulated Hemorrhage. Shock. 2016 Sep;46(3 Suppl 1):74-82.

68. Ross AJ, Medow MS, Rowe PC, Stewart JM. What is brain fog? An evaluation of the symptom in postural tachycardia syndrome. Clinical Autonomic Research. 2013;23(6):305-11.

69. Medow MS, Stewart JM. The postural tachycardia syndrome. Cardiol Rev. 2007 Mar-Apr;15(2):67-75.

70. Zubac D, Reale R, Karnincic H, Sivric A, Jelaska I. Urine specific gravity as an indicator of dehydration in Olympic combat sport athletes; considerations for research and practice. European journal of sport science. 2018;18(7):920-9.

71. Arnold AC, Ng J, Raj SR. Postural tachycardia syndrome - Diagnosis, physiology, and prognosis. Auton Neurosci. 2018 Dec;215:3-11.

72. Sheldon RS, Grubb BP,2nd, Olshansky B, Shen WK, Calkins H, Brignole M, et al. 2015 Heart Rhythm Society Expert Consensus Statement on the Diagnosis and Treatment of Postural Tachycardia Syndrome, Inappropriate Sinus Tachycardia, and Vasovagal Syncope. Heart Rhythm. 2015 Jun;12(6):e41-63.

73. Koziol-McLain J, Lowenstein SR, Fuller B. Orthostatic vital signs in emergency department patients. Ann Emerg Med. 1991;20(6):606-10.

74. Rosen DS. Physiologic growth and development during adolescence. Pediatr Rev. 2004 Jun;25(6):194-200.

75. Chavarro JE, Watkins DJ, Afeiche MC, Zhang Z, Sánchez BN, Cantonwine D, et al. Validity of self-assessed sexual maturation against physician assessments and hormone levels. J Pediatr. 2017;186:172,178. e3.

76. McKenzie, K., Murray, A., Freeston, M., Whelan, K., & Rodgers, J. (2019). Validation of the Revised Children's Anxiety and Depression Scales (RCADS) and RCADS short forms adapted for adults. Journal of Affective Disorders, 245, 200-204.

78. Singer W, Sletten DM, Opfer-Gehrking TL, Brands CK, Fischer PR, Low PA. Postural tachycardia in children and adolescents: what is abnormal? J Pediatr. 2012;160(2):222-6.

79. Skinner JE, Driscoll SW, Porter CJ, Brands CK, Pianosi PT, Kuntz NL, et al. Orthostatic heart rate and blood pressure in adolescents: reference ranges. J Child Neurol. 2010;25(10):1210-80. Oppliger RA, Magnes SA, Popowski LA, Gisolfi CV. Accuracy of urine specific gravity and osmolality as indicators of hydration status. Int J Sport Nutr Exerc Metab. 2005;15(3):236-51.

81. Sommerfield LM, McAnulty SR, McBride JM, Zwetsloot JJ, Austin MD, Mehlhorn JD, et al. Validity of Urine Specific Gravity When Compared With Plasma Osmolality as a Measure of Hydration Status in Male and Female NCAA Collegiate Athletes. J Strength Cond Res. 2016 Aug;30(8):2219-25.

82. Stand AP. Exercise and fluid replacement. Med Sci Sports Exerc. 2009;39(2):377-90.

83. Sawka M, Burke LM, Eichner R, Maughan RJ, Montain SJ, Stachenfeld NS. Exercise and Fluid Replacement. American College of Sports Medicine. 2007;39(2):377-340.

84. Armstrong LE, Maresh CM, Castellani JW, Bergeron MF, Kenefick RW, LaGasse KE, et al. Urinary indices of hydration status. Int J Sport Nutr. 1994;4(3):265-79.