Waking up to weight: The meditational role of sympathovagal balance in the relation between inadequate sleep and childhood obesity

Denise Christina Jarrin

A Thesis

In the Department

Of

Psychology

Presented in Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy (Psychology) at

Concordia University

Montréal, Québec, Canada

August 2012

CONCORDIA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared

By: Denise C. Jarrin

Entitled: Waking up to weight: The meditational role of sympathovagal balance in the relation between inadequate sleep and childhood obesity

and submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy (Psychology)

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final examining committee:

Dr. Richard DeMont — Chair

Dr. Kristen Salomon — External Examiner

Dr. Nina Howe — External to Program

Dr. Carsten Wrosch — Examiner

Dr. Jean-Philippe Gouin — Examiner

Dr. Jennifer J. McGrath — Thesis Supervisor

Approved by

Chair of Department or Graduate Program Director

Dean of Faculty

Abstract

Waking up to weight: The meditational role of sympathovagal imbalance in the relation between sleep and obesity in children

Denise Christina Jarrin, Ph.D.

Concordia University, 2012

The overarching goal of the present dissertation was to examine whether sympathovagal imbalance, as measured by heart rate variability (HRV), was a pathophysiological mechanism in the relation between sleep and obesity. While mounting evidence suggests sleep plays a causal role in the development of obesity, the underlying pathogenic pathways are complex and unresolved. Experimental sleep deprivation studies demonstrate sympathovagal imbalance subsequent to inadequate sleep. Further, obese children exhibit sympathovagal imbalance, particularly at night, compared to non-obese children. The question remains whether sympathovagal imbalance plays a meditational role in the relation between sleep and childhood obesity. The present dissertation consists of four manuscripts.

The first two manuscripts addressed knowledge gaps regarding the methodology and psychometrics of HRV in children. Manuscript 1 investigated the correspondence of HRV indices across contemporary computer software programs. Using triplicate electrocardiogram data derived from identical data acquisition hardware, this study demonstrated strong to excellent correspondence for HRV indices, contingent on the selection of rigorous user-decisions and technical specifications. Manuscript 2 yielded normative HRV values from a large, population-based sample of 10-year-old children.

Developmentally-relevant covariates were also identified. Referent values and covariates for each HRV parameter are presented.

The last two manuscripts tested the associations across sleep, obesity, and sympathovagal imbalance. Manuscript 3 examined whether multiple sleep parameters were individually related with obesity, independent of sleep duration. The novel findings suggest that additional sleep dimensions, beyond sleep duration, may more precisely capture the influences that drive the negative link between sleep and childhood obesity. Manuscript 4 tested whether sympathovagal imbalance was a potential pathophysiological mechanism linking sleep and obesity. In a sample of children at-risk for obesity, sympathovagal imbalance helped explain the relation between inadequate sleep with central adiposity and body composition.

Overall, the present dissertation contributed new knowledge to the field regarding HRV methodology and psychometrics in children. Original findings also demonstrated that sympathovagal imbalance mediated the relation between sleep and childhood obesity. Two general limitations included the use of cross-sectional designs and subjective sleep measures. Future research should include experimental or longitudinal designs with objective sleep measures to test the temporal relation of sleep, sympathovagal imbalance, and childhood obesity.

ACKNOWLEDGMENTS

First and foremost, I would like to offer my sincerest appreciation and gratitude to my supervisor, Dr. Jennifer McGrath, Director of the Pediatric Public Health Psychology Laboratory (PPHP). She has played a vital role in my graduate training experience; challenging me to think more critically, helping me develop my writing skills, and providing support and motivation with not only her words of wisdom, but also those from fortune cookies. This dissertation would not have been completed without our weekly powwows, her outstanding editorial skills with detailed and constructive comments, and her enduring commitment to me and my research. She is a huge source of inspiration for me and I am so privileged to have had the opportunity to work with her.

Completing this work would have been all the more difficult were it not for my fellow PPHP lab mates whose support, encouragement, and daily source of laughter have helped me through this process. A special thanks goes to my statistical life saver, Sabrina "Sabbi" Giovanniello, my partner in crime editing countless ECG files and chef, Neressa Noel, to my singing partner, Simon Racicot, and to Giovanna Daniele. Thank you Natasha Hunt for your amazing work coordinating the Health Heart Project.

I devote my deepest gratitude to my parents and younger siblings for their unlimited love and support throughout all my endeavors. I owe so much to my family and there are no words to express how much I cherish and love you.

In conclusion, I recognize that this research would not have been possible without the financial assistance of FRSQ, CIHR, and Concordia University School of Graduate Studies and I also express my gratitude to those agencies.

DEDICATION

To my loving and supportive parents, Monica Fernanda and Jose Ricardo

To my little ones, Bibi, Julian, and Stephanie

Contribution of Authors

The manuscripts presented as part of my dissertation were based on larger ongoing studies with co-authors contributing differently in each manuscript. For all four manuscripts, Dr. Jennifer McGrath and I jointly conceived the aim and design of each study. My responsibilities as the first author included completing the background literature review and synthesis, statistical analyses and interpretation of data, and drafting of the manuscripts, in their entirety. I was primarily responsible for incorporating comments and feedback from co-authors on subsequent drafts, and submitting, revising, and resubmitting manuscripts for publication.

Dr. Jennifer McGrath, the second author on all manuscripts, supervised and carefully edited each manuscript, in her role as research supervisor, providing invaluable input and feedback throughout each phase of my dissertation. She granted approval for use of the Healthy Heart dataset and secured approval for the QUALITY and QLSCD datasets (manuscripts 1, 2, and 4). Dr. McGrath provided critical conceptual and statistical guidance and advice. She shared her psychophysiological expertise on data reduction, cleaning, and interpretation. Additionally, she contributed to the decision of selecting journals, as well as the revision and resubmission of manuscripts for publication.

For manuscript 1, data were from the QUALITY Cohort, conducted by Team PRODIGY at St. Justine Hospital, University of Montréal. I was responsible for data reduction and cleaning of the electrocardiogram (ECG) data, writing all manuscript drafts, conducting statistical analyses, and incorporating comments and feedback from co-authors. Sabrina Giovanniello, the senior data coordinator and third co-author, coordinated data management and verified the mathematical algorithms used for heart

rate variability (HRV). Dr. Paul Poirier, fourth co-author, reviewed the ECG recordings to diagnose cardiovascular psychopathology, read all manuscript drafts, and provided substantive comments. Dr. Marie Lambert, fifth co-author and the principal investigator of the QUALITY Cohort, obtained the grant to fund the study, secured data collection, and granted permission for data use. (Dr. Marie Lambert died February 2012; she is included posthumously as a co-author given her significant contributions to this manuscript.)

For manuscript 2, data were from the QLSCD and its pilot sample conducted by Drs. Richard E. Tremblay and Jean R. Séguin, under the auspices of the Institute du Québec. Sabrina Giovanniello coordinated data entry and management. All co-authors (Drs. Jennifer J. McGrath, Paul Poirier, Jean Séguin, Louise Séguin, Richard E. Tremblay, Gilles Paradis) read the initial draft, proposed additional statistical analyses, and provided suggestions and feedback on the manuscript. Dr. Jean Séguin provided critical information about the QLSCD design and methodology. Dr. Paul Poirier provided important information on the cardiovascular effects of medications and their classification.

For manuscript 3, data were from the Healthy Heart Project, an ongoing study at the Pediatric Public Health Psychology Laboratory, Concordia University, Montréal. Dr. Jennifer McGrath obtained the grant funding, designed the study, and collected the data. I was a co-investigator on the grant. I selected the sleep questionnaires to be incorporated into the larger study, contributed to writing the grant, and tested participants. Dr. Christopher Drake, a co-author, read and edited early drafts, giving valued input and suggestions regarding the implications of the study.

For manuscript 4, data were from the QUALITY Cohort. All co-authors (Drs. Jennifer J. McGrath, Paul Poirier, Gilles Paradis) read manuscript drafts and provided pertinent feedback. For this manuscript, I reviewed the background literature, wrote the initial drafts, incorporated comments from co-authors, ran statistical analyses, and selected the journal for submission.

TABLE OF CONTENTS

List of Tables

MANUSCRIPT 1

MANUSCRIPT 2

MANUSCRIPT 3

MANUSCRIPT 4

List of Abbreviations

ANS..... Autonomic Nervous System

BMI..... Body Mass Index

CSHQ.....Children's Sleep Habits Questionnaire

DEXA .....Dual Energy X-ray Absorptiometry

ECG.....Electrocardiogram

HF .....High Frequency

HRV .....Heart-Rate Variability

ln .....Natural log transform

LF..... Low Frequency

LF:HF ratio .....Low Frequency/High Frequency Ratio

pNN50..... Percent difference in successive Beat-to-Beat intervals greater than 50 ms

rMSSD ..... Square Root Mean Difference of Successive Beat-to-Beat intervals

RR..... Beat-to-Beat

SA .....Sinoartial

SDANN.....Standard Deviation of the Average R-R Intervals for each 5 min Segment

SDNN.....Standard Deviation of all Beat-to-Beat Intervals

SDNNI .....Average Standard Deviation of all R-R Intervals for each 5 min Segment

VLF ..... Very Low Frequency

GENERAL INTRODUCTION

Childhood Obesity

Childhood obesity has been identified as a global epidemic (Ebbeling, Pawlak, & Ludwig, 2002; Hossain, Kawar, & El Nahas, 2007) with high prevalence rates in both developed and developing countries (Wang & Lobstein, 2006). In Canada, between 1981 and 1996 overweight rates doubled in girls and tripled among boys (Tremblay, Katzmarzyk, & Willms, 2002). In 2004, 26% of youth were overweight or obese compared to 31% in 2005 (Shields, 2006; Statistics Canada, 2009). Overweight and obesity confer risk to many health conditions such as chronic diseases, disability, and even premature death (Freedman, Dietz, Srinivasan, & Berenson, 1999; Must & Strauss, 2000). Early precursors for cardiovascular diseases, metabolic syndrome, and chronic inflammation are evident among overweight and obese youth.

Further, childhood obesity *tracks* into adulthood (i.e., a tendency for obese children to remain obese adults), suggesting there is a stable risk trajectory (Freedman, Khan, Serdula, Dietz, Srinivasan & Berernson, 2005; Whitaker, Wright, Pepe, Seidel, & Dietz, 1997). As such, there is an unprecedented impending economic burden on national health care costs. In the United States, over 30% of pediatric hospitalizations are attributable to obesity and obesity-related co-morbidities (Allison, Fontaine, Manson, Stevens, & Vanltallie, 1999; Wang & Dietz, 2002). Consistent with the increase in obesity rates over the past three decades, health costs associated with obesity have tripled from 1971-81 to 1997-99, representing more than $127 million annually in North America (Wang & Dietz, 2002).

Sleep and Childhood Obesity

Mirroring these obesity trends, sleep duration has decreased among youth across decades (Iglowstein, Jenni, Molinari, & Largo, 2003). Short sleep duration is consistently associated with larger weight status, in a dose-response pattern, in both children and adolescents, regardless of how obesity is defined [e.g., body mass index (BMI), waist and hip circumference, percent body fat] or whether sleep is subjectively or objectively measured (e.g., actigraphy, polysomnography; c.f., Beebe et al., 2007; Chaput, Brunet, & Tremblay, 2006; Chaput & Tremblay, 2007; Snell, Adam, & Duncan, 2007). Based on longitudinal studies, shorter sleep duration increases the risk of obesity and or weight gain in youth (c.f., Carter, Taylor, Williams, & Taylor, 2012; Snell et al., 2006; Taveras, Rifas-Shiman, Oken, Gunderson, & Gillman, 2008). Toddlers as young as 2½ years old and school-aged children with short sleep duration show increased risk of being overweight or obese 3 to 5 years later (Lumeng et al., 2009; Reilly et al., 2005). Similarly, later bed- and rise-times in children have been prospectively linked with greater weight status three years later (Snell et al., 2007). Retrospectively, frequent sleep problems during childhood are associated with a twofold increase in risk of being obese during young adulthood (Al Mamun et al., 2007).

Taken together, the extant literature convincingly demonstrates the link between inadequate sleep and the development and maintenance of obesity (c.f., Cappuccio et al., 2008; Marshall, Glozier, & Grunstein, 2008; Nielsen, Danielsen, & Sørensen, 2011). Given that obesity is a significant risk factor for multiple physical and psychological pathologies as well as an economic burden, better understanding of the pathogenesis of

obesity is of great importance. However, the mechanisms underlying the association between inadequate sleep and obesity are not fully understood.

Potential Pathogenic Pathway Linking Sleep and Childhood Obesity

Some researchers have postulated that reduced sleep duration may be considered a stressor that leads to adverse modifications in several critical systems in the body. The *allostatic load* theory refers to the wear and tear on the body and has been used to explain the pathophysiological mechanisms underlying the relation between short sleep duration and weight gain (McEwen, 2002, 2006). During normal sleep and wake cycles, the neuroendocrine, immune, and autonomic nervous system adapt and change to maintain homeostasis or *allostasis* (McEwen, 2002, 2006). These systems reflect a highly interconnected network and even small changes in production (e.g., hormones) can affect the entire network. When sleep is reduced, changes within the network occur and an allostatic overload develops (Danese & McEwen, 2012; McEwen, 2006). If this allostatic overload is prolonged due to chronic sleep loss, it can have behavioural, neurobiologic, and physiologic repercussions (McEwen, 2006; Speigel, Leproult, & Van Cauter, 1999; Spiegel et al., 2004; Van Cauter et al., 2007).

Although researchers have started to investigate the relation between sleep and obesity, there is still a paucity of information aimed at elucidating the underlying pathophysiological mechanisms. Experimental sleep deprivation studies with adults provide valuable insight into potential mechanistic pathways linking sleep and obesity, including energy expenditure, metabolism, insulin sensitivity, and hormones involved in appetite regulation (i.e., leptin, ghrelin; Spiegel et al., 1999, 2004). Another putative

pathophysiological mechanism is autonomic dysfunction, indexed by sympathovagal imbalance.

Sympathovagal Balance

The autonomic nervous system (ANS) is responsible for homeostasis in the body, vital organs (e.g., lungs, kidneys, pancreas), endocrine and exocrine glands, as well as the cardiovascular system (Furness, 2006; Goldberger, 1999). The ANS is comprised of two branches: the parasympathetic nervous system and the sympathetic nervous system. The parasympathetic and the sympathetic nervous systems work in balance on almost every organ of the body with differing yet, complementary effects. The parasympathetic nervous system is derived from the cranial nerves and spinal cord and is associated with the conservation of energy, allowing the body to "rest and digest" (Berntson et al., 1997; Goldberger, 1999; Snitker, Macdonald, Ravussin, & Astrup, 2000). Activation of the parasympathetic nervous system involves a reduction in heart rate, cardiac output, and blood pressure and facilitates digestion and restores energy (Goldberger, 1999; Snitker et al., 2000). In the parasympathetic nervous system, acetylcholine is the neurotransmitter responsible for many of these effects on the body, especially slowing heart rate (Berntson et al., 1997).

The sympathetic nervous system also derives from the spinal cord and prepares the body for the "fight or flight" response (Snitker et al., 2000). This involves vasoconstriction (reducing blood flow), metabolic changes of fat and glucose, higher blood pressure, cardiac output, and heart rate (Snitker et al., 2000). In the sympathetic nervous system, sympathetic neurotransmitters, such as the catecholamines epinephrine

and norepinephrine, increase cardiovascular functioning when stimulated (i.e., heart rate; Berntson et al., 1997).

Because the ANS controls the frequency at which the sino-atrial (SA) node, the pacemaker of the heart, triggers the initiation of each heart beat, heart rate variability (HRV) has emerged as a simple, reliable, and noninvasive technique to evaluate autonomic function and balance (Berntson et al., 1997, Task Force, 1996). HRV represents the continuous physiological variations between consecutive heartbeats. It expresses the total amount of oscillations of instantaneous heart rate and intervals between QRS complexes of normal sinus depolarizations (described below).

HRV is recognized as a valuable quantitative marker of sympathovagal balance, characterized as the overall balance between the sympathetic and parasympathetic activity of the autonomic state (Goldberger, 1999). While a healthy sympathovagal balance reflects a steady balance between the branches of the ANS, sympathovagal *imbalance* is characterized by reduced parasympathetic and or elevated sympathetic input (Goldberger, 1999). Sympathovagal imbalance, and consequent cardiovascular autonomic dysfunction (Messerli, Nunez, Ventura, & Snyder, 1987), is implicated in the pathophysiology of a number of cardiac and non-cardiac diseases, as well as mortality (e.g., Berntson et al., 1997; Task Force, 1996; Thayer & Lane, 2007).

HRV parameters are derived from an ECG, an electric signal waveform that can be used to discriminate and quantify normal and abnormal beat-to-beat changes in the heart between the two branches of the ANS (Berntson et al., 1997; Task Force, 1996). There are five points (P, Q, R, S, T) on the ECG waveform. These points normally follow a specific sequence and correspond to distinct events occurring in the heart. The

P-point represents the contraction of the heart's atria pumping the blood into the heart's ventricles. The time it takes for the heart's ventricles to fill up with blood is represented by the P-Q interval. Then, the electrical signal divides into the right and left branches on the heart's septum. This is depicted by the Q-point. The electrical signal leaves the right and left bundle branches via the Purkinje fibers and spreads rapidly across the ventricles causing them to contract. The left ventricle contracts an instant before the right ventricle and is represented by the R-wave. The number of R-waves per minute is an estimate of the heart rate for that minute (Berntson et al., 1997). The S-point represents the contraction of the heart's right ventricle. Finally, the relaxation of the heart's ventricles is denoted by the T-point and then the cycle continues.

Although there are numerous techniques to quantify HRV, the two most commonly and widely used approaches are time- and frequency-domain (power spectral) analyses (Berntson et al., 1997; Task Force, 1996). HRV time-domain methods are based on statistical calculations derived either from the direct measurement of RR intervals or from the differences between successive RR intervals (Task Force, 1996). Time-domain indices provide information on the variability of the heart rate fluctuations, however, they are not identical, and each index provides unique information. For instance, the standard deviation of all RR intervals (SDNN) broadly captures total HRV (Task Force, 1996). Further, the proportion of heart beats where the change from one beat to the next is > 50 ms (pNN50) and the average change in inter beat interval between beats (square root mean difference of successive RR intervals; rMSSD) are two time-domain variables known to reflect parasympathetic activity and are inversely correlated with sympathovagal imbalance (Kleiger, Stein, Bosner, & Rottman, 1992).

Using spectral techniques, such as the fast Fourier transform (FFT), HRV frequency-domain analyses identify and separate the variance in RR intervals into their underlying components at frequency-specific oscillations (Berntson et al., 1997; Berntson, Quigley, & Lozano, 2007; Task Force, 1996). Frequencies are categorized into power bands that reflect different branches of the cardiac ANS, including low frequency (LF) and high frequency (HF). Based on pharmacological blockade studies, LF is influenced by both sympathetic and parasympathetic inputs, while HF is influenced by only parasympathetic inputs (Akselrod et al., 1981; Berntson et al., 1997, 2007; Task Force, 1996). While HRV parameters exist that reliably reflect parasympathetic input (e.g., HF, rMSSD, pNN50), no HRV variable shows exclusive sympathetic input (Berntson et al., 1997, 2007). As such, the ratio between LF and HF has been proposed to provide an index of the relative balance between sympathetic and parasympathetic inputs on the cardiovascular system (i.e., sympathovagal balance; Goldberger, 1999; Malliani, Pagani, & Lombardi, 1994).

Sympathovagal Imbalance and Sleep

Experimental studies depriving participants of sleep have found significant increases in sympathetic activation, compared to non-sleep deprived individuals. Under conditions of sleep deprivation, adults show elevations in heart rate, systolic (SBP) and diastolic blood pressure (DBP; Lusardi, Mugellini, Preti, Zoppi, Derosa, & Fogari, 1996; Tochikubo, Ikeda, Miyajima, & Ishii, 1996), and norepinephrine and epinephrine concentrations (Irwin, Thompson, Miller, Gillin, & Ziegler, 1999; Tochikubo et al., 1996). Studies that assess HRV also find significant increases in sympathovagal

imbalance (e.g., decreased parasympathetic functioning) during the early morning and afternoon following chronic partial sleep loss.

Sympathovagal Imbalance and Childhood Obesity

Interestingly, sympathovagal imbalance has been prospectively linked with the development of obesity in children (Graziano, Calkins, Keane, & O'Brien, 2011). Several studies report sympathovagal imbalance (as measured by HRV) among obese children and adolescents, compared to their healthy-weight counterparts (c.f., Kaufman et al., 2007; Nagai & Moritani, 2004; Martini et al., 2001; Riva et al., 2001). Interestingly, sympathovagal imbalance is observed during the day, but is especially prominent during nocturnal hours (Martini et al., 2001; Riva et al., 2001), even after adjusting for duration of obesity, snoring, and sleep apnea (Rabbia et al., 2003). These studies provide credible support for the association between sympathovagal imbalance and childhood obesity.

Sympathovagal Imbalance as a Pathogenic Pathway

Thus, one potential pathogenic mechanism underlying the association between sleep and obesity is sympathovagal imbalance. First, there is strong evidence linking short sleep duration and obesity. Research findings have demonstrated that reduced sleep duration predicts future weight gain and obesity in children and adolescents. Second, based on experimental studies, sleep loss has been proposed as a causal risk factor in the development of sympathovagal imbalance, such that reduced sleep alters autonomic modulation following nights of inadequate sleep. Third, studies demonstrate a consistent link between sympathovagal imbalance and obesity. Indeed, a recent prospective study found that decreased parasympathetic activation was an independent predictor of childhood obesity 5.5 years later (Graziano et al., 2011). Taken together, evidence

suggests sympathovagal imbalance may be a potential pathway that mediates the relation between sleep and obesity.

Study Aim for Manuscript 1. HRV is a well-established and widely used indicator of the flexibility and balance of the ANS (Task Force, 1996). One reason for its popularity is due to technological advancements in ambulatory ECG monitoring over the past few decades (Nunan, Sandercock, & Brodie, 2010). Concurrent advancements in the signal processing software programs used to analyze, clean, and interpret the ECG data have also contributed to the increased use of HRV in the field (Task Force, 1996). However, despite its widespread use, gaps of knowledge continue to exist concerning important details on the methodological decisions related to signal preprocessing specifications, algorithms, and interpolation methods used with each software program. While there is extensive data on the reliability of different ECG acquisition hardware (c.f., Dietrich et al., 2010; Pinna et al., 2007; Sandercock, Bromley, & Brodie, 2005), there is no available information on the reliability of commercially available signal processing software programs currently in use (Jung et al., 1996). Relatedly, more than a decade ago, the Task Force (1996) recommended that comparative studies in the HRV field be conducted. Given the extensive use of HRV within multidisciplinary settings, the aim of manuscript 1 was to examine the measurement fidelity of HRV indices derived from commercially available signal processing software programs typically used across diverse disciplines.

MANUSCRIPT 1:

Measurement Fidelity of Heart Rate Variability Signal Processing: The Devil is in the Details

Jarrin, D. C., McGrath, J. J., Giovanniello, S., Poirier, P., & †Lambert, M. (2012; in press). *International Journal of Psychophysiology*.

†Posthumous. Special acknowledgement goes to Dr. Marie Lambert (deceased February 20, 2012), for without her vision and dedication, Team PRODIGY and the QUALITY cohort would not exist.

Abstract

Heart rate variability (HRV) is a particularly valuable quantitative marker of the flexibility and balance of the autonomic nervous system. Significant advances in software programs to automatically derive HRV have led to its extensive use in psychophysiological research. However, there is a lack of systematic comparisons across software programs used to derive HRV indices. Further, researchers report meager details on important signal processing decisions making synthesis across studies challenging. The aim of the present study was to evaluate the measurement fidelity of time- and frequency-domain HRV indices derived from three predominant signal processing software programs commonly used in clinical and research settings. Triplicate ECG recordings were derived from 20 participants using identical data acquisition hardware. Among the time-domain indices, there was strong to excellent correspondence (ICC_{avg}=0.93) for SDNN, SDANN, SDNNi, rMSSD, and pNN50. The frequency-domain indices yielded excellent correspondence (ICC_{avg}=0.91) for LF, HF, and LF:HF ratio, except for VLF which exhibited poor correspondence (ICC_{avg}=0.19). Stringent user-decisions and technical specifications for nuanced HRV processing details are essential to ensure measurement fidelity across signal processing software programs.

1. Introduction

Heart rate variability (HRV) is an indicator of the total amount of oscillations of heart periods between consecutive QRS complexes of normal sinus depolarizations (RR intervals). Reduced HRV, suggested to reflect hyperactive sympathetic and/or hypoactive parasympathetic nervous system, has been implicated in the pathophysiology of a number of health outcomes including cardiac conditions such as myocardial infarction and coronary heart disease (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996; Liao, Carnethon, Evans, Cascio, & Heiss, 2002), hypertension, and non-cardiac conditions such as obesity, diabetes (Masi, Hawkley, Rickett, & Cacioppo, 2007), insulin resistance (Lindmark, Wiklund, Bjerle, & Eriksson, 2003), metabolic syndrome (Hemingway et al., 2005), dyspepsia (Lorena, Figueiredo, Almeida, & Mesquita, 2002), irritable bowel syndrome, anorexia nervosa (Mazurak et al., 2011), epilepsy (Ferri et al., 2002), anxiety (Friedman, 2007; Friedman & Thayer, 1998), and major depressive disorder (Nugent, Bain, Thayer, Sollers, & Drevets, 2011), as well as mortality (Camm et al., 2001; Gerristsen et al., 2001; Thayer & Lane, 2007). Significant developments in statistical, spectral, and geometric signal processing to automatically derive HRV parameters have led to their increased use in multidisciplinary settings. As such, many signal processing software programs have been created to analyze HRV data. These programs offer rapid, automatic analysis of output based on sophisticated signal processing techniques and algorithms that identify and measure various electrocardiograph (ECG)-derived variables from each cardiac cycle. While there are previous recommendations from the Task Force (1996) for comparative data across studies, there is a lack of systematic comparisons across

computer software programs used to derive traditional time- and frequency-domain HRV indices.

1.1 Heart Rate Variability

Traditionally, the autonomic nervous system (ANS) has been thought to be reciprocally balanced (i.e., as one branch of the ANS increases activity the other branch decreases activity); however, evidence suggests that parasympathetic and sympathetic outflows are distributed multidimensionally (Berntson, Cacioppo, & Quigley, 1991). As such, HRV and each of its components are particularly valuable quantitative markers that provide information on the flexibility and balance of the branches of the ANS based on heart period series (Berntson et al., 1997; Task Force, 1996).

Although heart period series can be construed from several physiological signals including photoplethysmography (Lu, Yang, Taylor, & Stein, 2009), continuous blood pressure recordings (Parati, Saul, Di Rienzo, & Mancia, 1995), doppler ultrasound techniques (Jezewski, Kupka, & Horoba, 2008), and microwave reflectometry (Mase, Nagae, Ito, & Komada, 2010), it is most typically derived from continuously recorded ECG signals. Many of these alternative physiological signals yield only approximate indicators of sympathovagal imbalance (Berntson et al., 1997). For example, ambiguous waveform morphology from distal photoplethysmographic records or continuous blood pressure recordings contribute to difficulty identifying accurate reference points (Berntson, et al, 1997). ECG recordings are preferred and considered a simple, noninvasive technique with clear waveform morphology, as the instantaneous ventricular depolarization yields the highest signal-to-noise ratio rendering a clearly delineated R-wave (Berntson et al., 1997; Lu et al., 2009; Task Force, 1996). Further, ECG recordings

provide proximal and reliable information on heart period series to quantify HRV and ultimately, evaluate autonomic function and balance.

Following data acquisition and audio-to-digital (A/D) conversion of raw ECG signals, HRV analysis is comprised of two major phases: signal preprocessing and automated analyses to derive HRV parameters (Berntson et al., 1997; Kligfield et al., 2007). Signal preprocessing incorporates accurately identifying QRS complexes and removing artifacts, while still preserving the integrity of the respiratory sinus rhythm. Artifacts may be attributable to movement (i.e., muscle activity), external electromagnetic signals (e.g., 50/60 Hz power lines), or technical problems (e.g., poorly fastened electrodes; Berntson et al., 1997; Berntson & Stowell, 1998). Failure to identify artifacts can lead to missing or additional QRS complex detections and minor contamination can increase error in HRV results by ~30% (Berntson et al., 1997; Berntson & Stowell, 1998; Xia, Odemuyiwa, Gill, Malik, & Camm, 1993). Signal preprocessing is influenced by both technical specifications (sampling rate, digital filters; Bailey et al., 1990; Mortara, 1977; van Bemmel, Zywietz, & Kors, 1990) and algorithms used for ECG pattern recognition and interpolation (e.g., feature extraction, beat selection; Bailey, Horton, & Itscoitz, 1974; Bonner & Schwetman, 1968; Pipberger, Stallman, & Berson, 1962). Detector algorithms can be based on heuristic derivative equations that identify discrete measurements or adaptive thresholds, for example, the increasing edge of the R-peak (Bonner, Crevasse, Ferrer, & Greenfield, 1972; Pryor, Russell, Budkin, & Price, 1969). Alternatively, they can be based on complex statistical algorithms that use linear or nonlinear filters, different transformations, or discriminant function analysis (Köhler, Hennig, & Orglmeister, 2003; Pan & Tompkins, 1985;

Romhilt & Estes, 1968). Interpolation algorithms, to replace missing or abnormal heart period series, include proximal, piecewise cubic Hermite, non-linear predictive interpolation, linear, and cubic spline interpolations (Kim, Kim, Lim, & Park, 2009; Lippman, Stein, & Lerman, 1994; Malik & Camm, 1995).

Automated analyses predominantly use linear analyses such as time- and frequency-domain methods to quantify HRV indices (Task Force, 1996). Other nonlinear analyses including fractal (e.g., detrended fluctuation analysis, power-law correlation; Pincus 1995; Richman & Moorman, 2000), symbolic dynamics (Porta et al., 2001; Voss et al., 2009), and complexity/entropy measures (e.g., approximate entropy, sample entropy, Shannon entropy, corrected conditional entropy, multiscale entropy), also exist (Moltana, Tobaldini, & Porta, 2012; Porta et al., 2001; Voss et al., 2009). Although nonlinear analyses provide quantitative information on the regularity and complexity of autonomic cardiovascular control, linear analyses are most commonly reported in the literature. Time-domain approaches are based on statistical calculations derived from the direct measurement of RR intervals (e.g., SDNN, SDANN, SDNNi) or from the differences between successive RR intervals (e.g., rMSSD, pNN50; Task Force, 1996). Methodological study designs partly guide how data are partitioned for cleaning and aggregating. Partitioning data into meaningful conditions (e.g., baseline vs. task), categories (e.g., day vs. night), or smaller segments due to signal quality (e.g., 2 hr segment vs. ten 20 min segments) interrupts the contiguous nature of the ECG signal. Further, data reduction decisions on the duration of analytical epochs (e.g., 1 vs. 5 min) to compute aggregated HRV indices across the epochs may yield different values. These

decisions have important implications and must be carefully considered, especially for time-domain variables.

Frequency-domain variables are based on spectral analysis of RR intervals (Lahiri, Prince, Kannankeril, & Goldberger, 2008). Power spectral density decomposes RR intervals into their fundamental frequency components and provides information on the distribution of power as a function of frequency. Spectral analyses can include parametric (autoregressive; Yule-Walker, Burg) or nonparametric methods (Fast Fourier Transform, FFT; Kim et al., 2009). FFT is most commonly used to calculate the maximum variability in heart period series, based on ranges of frequency-specific oscillations of the RR intervals that reflect different branches of the cardiac system (Lahiri et al., 2008; Spiers, Silke, McDermott, Shanks, & Harron, 1993).

Low frequency (LF) ranges from 0.04–0.15 Hz and reflects the aggregate influences of both sympathetic and parasympathetic branches of the ANS (Akselrod et al., 1981; Berntson et al., 1997); although, some researchers have suggested LF to be mainly of sympathetic origin (Malliani, Pagani, Lombardi, & Cerutti, 1991). High frequency (HF) ranges from 0.15–0.40 Hz and represents parasympathetic activity (Berntson et al., 1997; Pomeranz et al., 1985). Less studied frequencies include very low frequency (0.0033–0.04 Hz) and ultra low frequency (<0.003Hz) ranges; these are thought to be influenced by the renin-angiotensin system as well as thermoregulatory processes and circadian rhythms (Kitney, 1980; Taylor, Carr, Myers, & Eckberg, 1998). Importantly, default frequency bandwidths may differ across software programs leading to misinterpretation of the calculated HRV indices. For example, if HF was set

incorrectly to 0.12-0.40 Hz, results would actually include LF as well, and therefore, not solely represent the parasympathetic nervous system.

Another important decision for spectral analyses includes windowing. Spectral windowing involves the application of a window function, of a specified width, to shape the time portion of ECG data by overlapping waveform endpoints in a smooth, continuous way without sharp transitions to minimize edge effects that result in spectral leakage for better overall spectral resolution. Hamming or Hanning windows are commonly used due to their high quality frequency resolution and reduced spectral leakage (Bloomfield, 1976; Harris, 1978).

In the extant literature, research studies that use HRV report meager details on the methodological decisions related to signal preprocessing specifications, algorithms, and interpolation methods used. Time- and frequency-domain HRV indices are vulnerable to artifacts, missing data, temporal factors, and trends in RR intervals (Kim et al., 2009; Kim, Lim, Kim, & Park, 2007; Spiers, et al., 1993; Task Force, 1996), and are thus highly influenced by decisions for data reduction, artifact detection and removal, and technical specifications (e.g., digital filtering, sampling frequency, detector or interpolation algorithms, and windowing; Kim et al., 2009; Task Force, 1996; Welch, 1967).

Despite its extensive use, the comparability between standard computer HRV software programs has not been systematically evaluated. There is scant evidence of comprehensive comparisons to assess the fidelity of signal processing across multiple software programs. Of the only study to compare HRV signal processing programs, Jung and colleagues (1996) found time- and frequency-domain variables were not comparable

across four programs in widespread use at the time almost two decades ago. Jung attributed the large variability across programs to different technical specifications, including beat selection methods (e.g., best complex, time-coherent averaging, extraction), sampling frequency, interpolation, and algorithms.

1.3 Present Study

A significant challenge exists for researchers who want to compare or synthesize HRV results across studies. In a systematic review on short-term HRV measures, Nunan and colleagues (2010) found considerably large variations across studies, especially for frequency-domain variables. These discrepancies were attributed to differences in study design and methodology, as well as failure from authors to provide pertinent information on signal processing and data cleaning procedures. The importance of standardization across studies was reinforced by the Task Force (1996) guidelines in hopes to facilitate the exchange of knowledge, allow for comparative results across studies, and avoid conflicting data due to different technical and methodological approaches. Following these recommendations for standardization and interpretation of HRV measures, the purpose of the present study was to evaluate the measurement fidelity of HRV indices derived from three predominant signal processing software programs most commonly used in clinical and research settings among cardiologists, psychophysiologists, and other researchers across diverse disciplines (MARS, MindWare, Kubios). Using triplicate ECG data derived from identical data acquisition hardware, the comparability of HRV indices for time-domain (i.e., SDNN, SDANN, SDNNi, rMSSD, pNN50) and frequency-domain variables (LF, HF, LF:HF ratio) was tested.

2. Material and Method

2.1 Measures

2.1.1 ECG Data Acquisition

Twenty Holter tapes with raw ECG data were randomly chosen from an ongoing study of healthy youth participants between the ages of 8 and 11 (M_{age} = 9.93 years, *SD* = 1.02; 55% male). The complete research protocol is described elsewhere (Lambert et al., 2011). All ECG recordings were reviewed by a board-certified cardiologist; no cardiovascular pathology was identified (i.e., bradycardia, fibrillation, premature contraction). During the standardized protocol conducted in a hospital setting, continuous raw ECG data were acquired using the 8500 Marquette MARS Holter monitor (GE Marquette Medical Systems, Milwaukee, Wisconsin, USA), digitized (128 Hz), and recorded on a frequency modulated cassette recorder. The Holter monitor incorporated a quartz-derived, binary time channel that was automatically zeroed at the start of the recording. ECG acquisition began in the morning between 8 and 9 am and lasted approximately 2.5 hours.

ECG data was derived from a modified Lead II configuration using disposable, pre-gelled snap silver chloride electrodes. Electrode resistance was minimized (<10 kO) by precleaning the skin with rubbing alcohol swab. The active electrode (and its derivative/dZ) was placed on the right clavicle next to the sternum over the first rib between the two collarbones. The second electrode was placed on the left mid-clavicular line at the apex of the heart over the ninth rib. The ground electrode was placed near the lowest possible right rib cage on the abdomen. Additional dZ electrodes were placed over the right fourth intercostal space at the sternal edge, the fifth intercostal space at the

left axillary line, and on the sixth rib in the mid-clavicular line. To reduce possible violations of stationarity, the ECG acquisition procedure was standardized and kept consistent for all recordings (Berntson et al., 1997). The study was reviewed and approved by the St. Justine Hospital Institutional Review Board (#2040).

2.2 Procedure

2.2.1 Data Processing Procedure

ECG Holter tapes underwent identical processing procedures for each software program. Triplicate ECG data signals were derived from each of the 20 recordings. Each triplicate ECG recording was cleaned by a qualified investigator and independently auto scored with all three signal processing software programs strictly adhering to both Task Force (1996) guidelines and manufacturer specifications (described in detail below; see Table 1).

2.2.2 A/D Data Conversion

MARS. From the Holter tapes, ECG data files were downloaded and formatted into the MARS® Holter Analysis Workstation v.7.0 (Milwaukee, Wisconsin, USA).

MindWare and Kubios. ECG Holter tapes were converted and digitized into Waveform Audio (WAV) version using a high-grade contemporary dual capstan deck unit. WAV files were imported into shareware software for recording and editing audio files (Audacity® v.1.2; http:// audacity.sourceforge.net). The speed of the audio signal was resampled and the length, pitch, and frequency were optimized to yield clear high-quality ECG signals. Then, using a 4-channel high-level interface module in the BioNex 2SLT Chassis Assembly (MindWare Technologies Ltd., Columbus, Ohio, USA) and the Biolab 3.0 data acquisition software (16-bit A/D conversion) the resampled digital data

files were imported (sampled at 250ks/s rate), converted, and formatted into MindWare (MW) files, while preserving the integrity of the signal. One set of raw MW formatted data files were imported into MindWare® HRV Scoring Module v.3.0.17 (MindWare Technologies Ltd., Columbus, Ohio, USA). A duplicate set was converted into ASCII text files and imported into the Kubios® HRV v.2.0 (University of Eastern Finland, Kuopio, Finland; Niskanen, Tarvainen, Rantaaho, & Karjalainen, 2004). It is important to note that all software programs were used without applying any ad hoc custom-made routine changes (i.e., all default settings and specifications were maintained). The only exception was the adjustment of the default frequency bandwidths for LF and HF in MindWare; these were adjusted in accordance with the Task Force (1996) guidelines. Signal processing and default specifications are outlined below for each software program.

2.2.3 Data Cleaning

Beat-by-beat intervals with near millisecond measurement of continuous ECG data were required for data cleaning. Missed or unidentified R-peaks by each respective program's detector algorithm were manually relabeled (refer to Table 1; data cleaning section). In conjunction with each software program's automated cleaning procedure, pre-defined cleaning guidelines adhering to the recommendations in the expert committee report were used by a trained investigator to accurately discriminate QRS complexes (Berntson et al., 1997). If an R-peak was automatically detected, but upon visual inspection was not found to be accurate, ≥2 short inter-beat-intervals were added to retain the integrity of the heart period series. If an R-peak was not automatically detected, the following guidelines (in rank order) were applied: (1) RR interval distance from cleaned

ECG recording sample was measured, (2) R-peak was estimated from remaining data points, and (3) long R-peak were split into ≥2 equal RR intervals (Berntson et al., 1997).

2.2.4 ECG Signal Processing

MARS. Signal processing specifications for detector algorithms and interpolation methods were based on default settings (refer to Table 1). Detector algorithms require at least 5 min of data to calculate HRV indices (adjustable). Beat-by-beat visual inspection of the shape, trend, and length of each QRS complex were measured and identified based on template matching and standard Marquette algorithms for QRS labeling. ECG data was sampled at various rates resulting in QRS timing at different resolutions (1024 samples/300 s) and RR filtering was automatic (manual filter available). The removal of artifacts was based on a 20% change from the previous signal as a criterion (Kleiger, Miller, Bigger, & Moss, 1987). In cases where artifacts and excluded RR intervals were automatically filtered and identified as unreadable signals, the remaining acceptable beats were used to replace the data points via cubic spline interpolation method. At least four acceptable R-peaks were needed in order for spline interpolation to identify the continuous function between two middle R-peaks. If there was no data in the first segment (e.g., noise), then RR interval series were interpolated from the default heart rate of 70 bpm (adjustable).

For spectral analyses, trending, interpolation rate, interpolation method, and windowing options (e.g., window width and overlapping) were based on default settings. Heart period series were linearly detrended, tapered using a Hanning window, and processed by FFT periodogram spectrum method. Time-and frequency-domain

parameters were automatically calculated for each 5 min epoch across the entire data file. HRV parameters were then automatically averaged across the entire recording period.

MindWare. Signal processing specifications for detector algorithms could be manually overwritten, and included inter-beat-interval check and automated Minimum Artifact Deviation and Maximum Expected Deviation (MAD/MED) algorithm (Berntson, Quigley, Jang, & Boysen, 1990). For the present study, 5 min analytical epochs and both detector algorithms were applied. R-peak detection was based on default digital low- and high-pass filters set within appropriate frequency ranges (0.05 and 35 Hz, respectively; adjustable)**.** Frequency bandwidths were user-defined for LF (0.04-0.15 Hz) and HF (0.15-0.40 Hz). Beat-by-beat visual inspection of the shape, trend, and length of each QRS complex data was displayed on a full graphical interface. ECG signals were sampled at 1000 Hz and RR filtering was automatic (manual filter available). RR intervals that were excluded due to unreadable signals or recognition error were replaced by cubic spline interpolation and resampled at a frequency of 33.33 Hz.

Spectral analyses were performed on a series of RR intervals and were first linearly detrended using a Hanning window and processed by FFT standard power spectrum method. All time- and frequency-domain variables were automatically calculated for each 5 min epoch and averaged across the entire recording period, except for SDANN and SDNNi, which were manually calculated using standard formulae (Task Force, 1996).

Kubios. Signal processing specifications for detector algorithms and interpolation methods were based on default settings (adjustable; refer to Table 1). Visual inspection of the beat-by-beat RR intervals were measured and identified based on

template matching and proprietary algorithms. The sampling frequency was based on beat-by-beat RR intervals and automatically filtered, where RR intervals were divided into 5 min non-overlapping segments. As recommended by Kubios, based on visual inspection using the graphical interface, an artifact correction level (range from none to very strong) was selected for each date file. Each correction level applies thresholds (very low: 0.45 s, low: 0.35 s, medium: 0.25 s, strong: 0.15 s, very strong: 0.05 s) that are scaled with a heart rate of 60 beats/min. Scaling is used to adjust for heart rate changes within the recording (i.e., higher heart rate applies greater thresholds). High-pass filters on RR interval series remove all baseline changes from the data file, and from this detrended data, any beats that exceed the respective thresholds are identified as artifacts and removed (M.P. Tarvainen, personal communication, March 21, 2012). Because data cleaning is limited to this gross categorization to detect artifacts, Kubios recommends that artifact correction level should not be selected blindly, but should include manual visual inspection and verification of the correction level selected within the graphical interface. Continuous heart period series were corrected by piecewise cubic spline interpolation method at the default rate of 4 Hz (adjustable). Using a window width of 256 s (window overlap of 50%; adjustable), samples were smoothed prior to detrending, tapered using a Hanning window, and processed by the Welch's periodogram method.

2.3 Analysis Plan

All data were entered and double-checked by the senior data coordinator and analyzed with IBM SPSS Statistics 20 software (SPSS, Inc., Chicago, IL). Data were kept continuous and checked for normality and linearity using boxplots and histograms.

Assumptions of additivity, homoscedasticity, uncorrelated error, and random selection of participants were tested (Shrout & Fleiss, 1979).

To assess measurement fidelity across the three software programs, Intraclass Correlation Coefficients (ICC), Pearson correlation coefficients, and Bland-Altman statistical methods were computed. An ICC is a measure of agreement between two or more evaluation methods on the same data that allows for fixed and random effects. Data are assumed to be parametric (continuous and normally distributed). ICCs typically range from 0 to 1, but can exceed -1 or 1, which may be attributable to patterns of negative and positive correlations among the methods, limited variance in the data matrix, or no correlations among methods (Lahey, Downey, & Saal, 1983). ICCs are categorized as very poor (0-0.2), fair (0.3-0.4), moderate (0.5-0.6), strong (0.7-0.8), or excellent (0.9-1.0; Shrout & Fleiss, 1979). ICCs are deemed advantageous over bivariate correlation coefficients as they represent the correspondence between two or more methods, and importantly, adjust for the effects of the scale of measurement. In other words, ICCs account for differences in rank order and mean differences between methods (data centered and scaled using pooled mean across methods and standard deviation), while correlations only account for rank order differences (data centered and scaled using each method's own mean and standard deviation). Nevertheless, Pearson correlation coefficients were computed for comparison purposes. Analysis of variance (ANOVA) was also used to test omnibus mean differences of the HRV parameters, followed by contrasts using paired samples *t*-tests.

The Bland-Altman method is used to graphically display the degree of agreement between two techniques on a continuous variable and to assess possible constant and

proportional biases (Bland & Altman, 1986, 2003). The differences in the measurements are plotted against the mean values of these measurements. If 95% of the differences fall within the limits of agreement (1 SD) there is no systematic variation across programs (Bland & Altman, 1986, 2003). To detect constant bias (i.e., the average discrepancy between methods of measurements), the mean bias and limits of agreement are used and should be close to zero. To detect proportional bias, visual inspection of the plotted graphs is commonly used; however, standardized β values can be used to test whether the slope is significantly different than zero (i.e., when mean values are regressed onto mean differences).

3. Results

The average length of the 20 ECG recordings was 131 min (SD = 46). All ECG recordings were inspected manually to review peak detection and to identify and remove artifacts. Manual editing took approximately 25 min per ECG recording. Recordings were found to be of excellent quality; over 90% of data were analyzable, artifact time did not exceed 1,500 s (5.2%), and no recordings were found to exceed 20% noise or ectopic beats.

ICCs were computed to compare the fidelity of HRV scoring across the software programs (see Table 2). Among the time-domain indices, there was strong to excellent correspondence across all software programs for SDNN ($ICC_{avg} = 0.96$; $r_{avg} = 0.97$), SDANN ($ICC_{avg} = 0.93$; $r_{avg} = 0.88$), SDNNi ($ICC_{avg} = 0.96$; $r_{avg} = 0.97$), rMSSD ($ICC_{avg} = 0.80$; $r_{avg} = 0.93$), and pNN50 ($ICC_{avg} = 0.98$; $r_{avg} = 0.99$). Among the frequency-domain indices, there was excellent correspondence across all software programs for LF ($ICC_{avg} = 0.90$; $r_{avg} = 0.94$), HF ($ICC_{avg} = 0.91$; $r_{avg} = 0.96$), and LF:HF ratio ($ICC_{avg} =$

0.95; r_{avg} = 0.93). However, VLF exhibited poor correspondence (ICC_{avg} = 0.19); these findings may be largely attributable to the significant mean level differences observed across software programs (see Table 3). Pearson coefficients revealed moderate correlations for VLF when mean level differences are not considered (r_{avg} = 0.83).

Bland-Altman plots and analyses were conducted to assess measurement fidelity for each HRV parameter paired by software programs (30 plots not depicted for parsimony). For each HRV parameter, the differences between each of the paired software programs were plotted against the average values of these measurements. Consistent with the recommendations outlined by Bland and Altman (1986, 2003), data were log-transformed prior to the calculation of limits of agreement when heteroscedasticity was present. There was no evidence of constant or proportional biases for any of the time-domain variables: SDNN ($Bias_{avg}$ = 0.02, [Limits of $Agreement_{avg}$ = -0.03, 0.08]; β_{avg} = -0.07), SDANN ($Bias_{avg}$ = 0.04, [-0.05, 0.14]; β_{avg} = 0.05), SDNNi ($Bias_{avg}$ = 0.03 [-0.06, 0.13]; β_{avg} = -0.16), rMSSD ($Bias_{avg}$ = 0.09 [-0.00, 0.19]; β_{avg} = 0.07), and pNN50 ($Bias_{avg}$ = 0.07 [-0.09, 0.25]; β_{avg} = -0.06). Similarly, no constant or proportional biases were observed for the frequency-domain variables: VLF ($Bias_{avg}$ = 0.70 [0.43, 0.96]; β_{avg} = -0.00), LF ($Bias_{avg}$ = 0.10 [-0.02, 0.22]; β_{avg} = -0.19), HF ($Bias_{avg}$ = 0.13 [-0.01, 0.29]; β_{avg} = 0.22), and LF:HF ratio ($Bias_{avg}$ = 0.10 [-0.02, 0.22]; β_{avg} = -0.11). Altogether, the results from the ICCs and Bland-Altman analyses were congruent.

4. Discussion

Recent advances in the automated analyses of HRV offers an accessible and unique approach for quantifying the effects of sympathetic and parasympathetic branches of the ANS. Despite evidence of the reliability of HRV parameters across different

recording devices, measurement protocols, and maneuvers (Dietrich et al., 2010; Faulkner, Hathaway, & Tolley, 2003; Pinna et al., 2007; Sandercock, Bromley, & Brodie, 2004, 2005; Sandercock, Shelton, & Brodie, 2003), there is no available information on the fidelity of commercially available signal processing software programs currently in use (Jung et al., 1996). The aim of the present study was to evaluate the measurement fidelity of HRV indices derived from three commonly used signal processing software programs.

Following stringent standardization (i.e., data collection, processing, and cleaning), excellent measurement fidelity for time-domain variables (e.g., SDNN, SDANN, SDNNi, rMSSD, pNN50) was observed across programs. Excellent correspondence was also observed for LF, HF, and LF:HF ratio. Poor correspondence was found for VLF; however, examination of the Pearson correlation indicates a moderate association across software programs. The excellent comparability for HRV variables is likely attributable to similar signal processing techniques and pivotal user-defined specifications across software programs (i.e., R-peak detection algorithm, identical analytical epoch length). For instance, the use of algorithms parallel to the Pan-Tompkins for the recognition of QRS complexes was apparent across all software programs (Pan Tompkins, 1985). As such, the ECG signal is passed through an automated low- and high-pass filter to remove noise. After filtering, the signal passes through derivative (to obtain QRS slope), squaring (to emphasize higher frequencies), and window integration phases (to identify waveform patterns), where lastly, a threshold method is applied and R-peaks are detected. As for the frequency-domain variables, windowing options (i.e., width and overlap) and frequency bandwidths must also be

taken into consideration (Task Force, 1996). In the present study, all software programs applied linear detrending method, cubic spline interpolation, with similar windowing (Hamming and Hanning) and spectrum methods (Periodogram and Welch's periodogram).

User-defined data reduction decisions can have significant implications on the automatic analysis of HRV parameters. Short analytical epochs (e.g., 1 min) and recording durations (< 18 hrs) may fail to capture the full spectrum of components or underlying circadian rhythms (Massin, Maeyns, Withofs, Ravet, & Gérard, 2000; Task Force, 1996). For example, the lowest frequency that can be assessed with 1 min is 0.016 Hz (G. Berntson, personal communication, December 15, 2011), indicating that it does not quantify the full spectrum of VLF components. Thus, to capture data at the lowest frequency, larger analytical epoch durations must be chosen (e.g., 3 to 5 min; Task Force, 1996). Further, the established physiological components and frequency bandwidth ranges are less well-defined for VLF, as compared to HF and LF (Berntson, Cacioppo, & Quigley, 1994; Cacioppo et al., 1994). Analytical epoch length, recording durations, and frequency bands should be consistent when making comparisons of HRV.

Given that technical specifications for data cleaning vary across programs, it is essential to know whether programs allow for manual inspection (i.e., some permit simultaneous automatic and manual cleaning and editing decisions). For example, MindWare offers users much flexibility to visually inspect and adjust RR fiducial points and identify important event markers (e.g., during tasks). In contrast, Kubios suggests visually inspecting data and applying an automated artifact correction based on gross categorization levels (e.g., low). Given the sensitivity of certain HRV parameters (e.g.,

rMSSD; Salo, Huikuri, & Seppanen, 2001), the level of gross artifact correction may be appropriate for some variables, while less appropriate for others. Taken together, these specific user-defined decisions likely account for the exceptional correspondence across software programs.

The present study yields original findings indicating the robust comparability for HRV across commonly used signal processing programs. While proprietary detector and interpolation algorithms are typically set, the excellent correspondence across software programs is largely attributable to seemingly nuanced, yet significant decisions. These include decisions related to the modification of particular user-defined and default settings (e.g., analytical epoch duration, frequency-bandwidths), use of cleaning tools (e.g., selection of appropriate artifact correction level), and inherent procedures in each software program (e.g., removing partial inter-beat intervals prior to data analysis).

Prior to selecting signal processing software, the conceptualization and understanding of HRV physiological indices is imperative. There is growing interest and advancements using neuroimaging techniques (e.g., functional magnetic resonance imaging) to better understand neurobiological (brain-body) interactions (c.f., Gianaros & Sheu, 2009; Gianaros, Van Der Veen, & Jennings, 2004). For example, HF has been associated with activity within the ventral anterior cingulate (Matthews, Paulus, Simmons, Nelesen, & Dimsdale, 2004), posterior cingulate cortex (O'Connor et al., 2007), amygdala, periaqueductal gray, and the hypothalamus in response to somatosensory stimuli (Gray et al., 2009) and isometric exercise (Napadow et al., 2008). Given the evidence of an association between the brain and the ANS (i.e., parasympathetic and sympathetic activity), these promising research directions

underscore the importance of purposeful and informed selection of HRV parameters. Consider, if the research question centers around assessing parasympathetic activity, it is necessary to select HRV parameters that validly reflect this activity in the ANS (e.g., HF, pNN50, rMSSD; Task Force, 1996). This in turn will directly impact decisions related to methodological design and measurement issues, including the recommended recording length to capture parasympathetic activity (e.g., 1 min), and an effort to minimize non-stationarity across conditions and participants, particularly for frequency-domain variables (Task Force, 1996). Other decisions may include whether recordings will be partitioned by task or interval (e.g., baseline vs. task, sleep vs. wake state). Similar issues were eloquently raised in a thorough review by Nunan and colleagues (2010) investigating normative HRV values from short-term recordings in healthy adults. Taking these pivotal methodological decisions into consideration will facilitate comprehensive systematic comparisons across studies and further advance the field.

4.1 Post-hoc Observations

Kubios. Several researchers report using an alternate strategy to clean data prior to using Kubios by deleting aberrant inter-beat intervals less than 300 and greater than 1200 ms (c.f., Capa, Cleeremans, Bustin, & Hansenne, 2011; Li et al., 2009; Rodríguez-Colón, Bixler, Li, Vgontzas, & Liao, 2011; Timonen et al., 2006). Data were re-analyzed with Kubios after applying this commonly reported data cleaning strategy. Post-hoc analyses revealed no significant differences across software programs for both time- and frequency-domain variables when this data cleaning strategy was applied (data not shown for parsimony).

4.2 Strengths and Limitations

The first limitation of the present study was the use of short- rather than long-term recordings (i.e., 3 vs. 24-hours). However, many studies typically record for similarly short durations. In keeping with the recommendations by the Task Force (1996), the present study adhered to a strict protocol for the acquisition, recording, collection, cleaning, and analyses of the data under standardized settings to minimize measurement error.

The second limitation was the use of only three software programs for comparison. These programs were purposely selected due to their ubiquitous use within clinical and research settings among psychophysiologists, cardiologists, and general researchers. Nevertheless, it is important to recognize there are additional commercially available as well as investigator-created software programs; however, their inclusion was beyond the scope of the present study. Future comparisons should be conducted using other software programs.

The third limitation was the assessment of only time- and frequency-domain variables. Geometric (e.g., triangular shapes of Lorenz plots) and nonlinear methods (e.g., detrended fluctuation analysis, approximate entropy) can also be used to analyze HRV (Pincus, 1995; Porta et al., 2001, 2007; Richman & Moorman, 2000; Task Force, 1996; Voss et al., 2009). However, these methods largely depend on the precision of equipment (i.e., obtain appropriate number of RR intervals), recording length (i.e., preferably 24 hours for geometric methods), and capability of these advanced analyses in software programs. Time- and frequency-domain variables are traditional HRV parameters reported in the majority of studies; thus, the comparability of these specific

parameters were deemed particularly important to inform future comparisons and syntheses across published studies (Task Force, 1996).

Lastly, all ECG recordings were derived from a Holter monitor manufactured by GE Marquette, the same manufacturer of MARS software program. However, it is unlikely that having a common manufacturer created any bias for the MARS software analyses. In fact, a major strength of the present study was the use of identical ECG recordings in triplicate for the three software programs. In other words, each software program analyzed the exact same ECG data. Thus, these findings are generalizable to the scenario quite common in research and clinical settings when hardware and software manufacturers differ.

4.3 Recommended Strategies

Although there are an increasing number of studies investigating HRV, the methodological, measurement, and technical specifications are not consistently applied in the field. These discrepancies add confusion to the interpretation of HRV and hinder advancement in the field because findings cannot be synthesized. Hence, to maximize measurement fidelity researchers must be cognizant of these subtle, yet pivotal fine details when using software programs. Two recommended strategies are provided.

Equipment and Software Specifications: Differences across user-defined choices and specifications of software programs may contribute to HRV discrepancies across studies. Researchers should report specific information about the recording equipment, signal (pre)processing software, software applications, and features selected (e.g., sampling rate of 250-500 Hz or higher, RR interval filter characteristics, R-peak detection and interpolation algorithms). Further, if frequency-domain variables are

analyzed, additional information on the spectral decomposition method, spectral windowing, window overlap, and the defined range of frequency bandwidths should be specified.

Data Reduction and Cleaning: Data reduction and cleaning decisions prior to HRV analysis (either by default or adjustable settings) should be explained and justified. For example, because the removal of erroneous beats or the unintentional removal of normal beats may affect the analysis and the comparability of HRV parameters (Berntson et al., 1997; Berntson & Stowell, 1998; Xia et al., 1993), the rationale for any exclusion criteria should be clearly stated. Furthermore, to facilitate systematic comparisons and synthesis of data, it is important to provide complete information on data reduction decisions. These include justification for how the data were segmented or partitioned for aggregating (e.g., conditions, tasks, control vs. clinical groups), cleaning (e.g., duration of analytical epochs), and analyzing (e.g., night vs. day). Complex study designs (e.g., multiple discreet intervals) may warrant use of software that permits greater flexibility for user-specifications and manual cleaning (i.e., Mindware). Regardless of what equipment or software is used, movement artifacts, technical failure, or poor data quality can seriously contaminate the integrity of the data. Despite the crucial task of manually cleaning data, specific procedures and decision rules are rarely reported. Basic information on the RR interval error identification, removal, criteria (e.g., thresholds), and correction procedures should be provided.

4.4 Future Research

Future studies should assess the measurement fidelity of time- and frequency-domain HRV variables with longer recordings (e.g., 24 hours), under differing conditions

(e.g., day vs. night), and in response to standardized challenges (e.g., stress testing; cold pressor reactivity). Additional geometric methods (i.e., HRV triangular index) should also be considered. Further, comparisons could be made for HRV parameters derived from different recording hardware and then analyzed with different software programs, as this would be a more ecologically valid reflection of the diverse practices across the research field. The contribution of the present study highlights the importance of providing sufficient detail about the signal acquisition hardware, the signal processing software, and the overall procedures used to derive HRV variables. Lastly, given that guidelines to specify standard definitions of HRV terms and measurement methodology were published almost two decades ago (e.g., Task Force, 1996; Berntson et al., 1997), there is merit in the proposal of updating the critical considerations in HRV analyses (e.g., Nunan et al., 2010).

4.5 Conclusion

The present study demonstrated that stringent decisions and specifications for subtle details are instrumental in the acquisition of excellent measurement fidelity across three commonly used HRV signal processing software programs. Specifically, signal processing, data cleaning, analysis, and interpretation specifications must be meticulously selected to enhance the precision of HRV data and should not be underestimated. Given the significance and value of comparing and synthesizing results across studies, it is crucial for researchers to understand and accurately report the technical specifications applied for HRV analyses.

Acknowledgements: Sincere thanks to the developers, technicians, and customer support staff of the investigated software programs for their extensive help, clarification, and detailed information provided (Kubios: Mika P. Tarvainen; Mindware: Gary Berntson, Marty Gillman, Greg Norman, Doug Schiefer, Dave Lozano, Gene Barbanera, Sean Canavan; MARS/GE Marquette Medical Systems: Jason Castillo, Pascal Langlois, Marilyn Manders). This work was made possible through funding support from the Canadian Institute of Health Research (CIHR) New Investigator Award (J. J. McGrath), Fonds de la Recherche en Santé du Québec Doctoral Fellowship (FRSQ) and Health Professional Student Research Award (D. Jarrin). The Holter recordings were obtained from the QUALITY Cohort, which is funded by CIHR, FRSQ, and the Heart and Stroke Foundation of Canada. The QUALITY Cohort was conducted by members of Team PRODIGY, an inter-university research team including Université de Montréal, Concordia University, Université Laval, McGill University, and INRS-Institut Armand Frappier.

Disclosure: There are no conflicts of interest to disclose. No software companies provided any compensation or financial support. All software was procured through standard procedures; no complimentary software access was provided. The use of the selected hardware and software programs does not necessarily constitute or imply their endorsement or agreement with the results of this study. The software companies were not involved in the study design, data analyses, data interpretation or manuscript writing and submission processes.

Table 1

System-dependent Specifications across Signal Processing Software Programs

	MARS (GE MARQUETTE)	MINDWARE	KUBIOS HRV	TASK FORCE
VERSION	● MARS Holter Analysis Workstation v7	● HRV v3.0.17	● Kubios HRV v2.0	
		ACQUISITION & CONVERSION		
IMPORT OPTIONS	● Raw ECG signals	● Raw ECG signals, BIOPAC (.acq) & conversion to Mindware format (.MW)	● Only RR Intervals	
INPUT FILES	● MARS software	● Mindware format (.MW)	● ASCII	
A/D RESOLUTION	● Not reported	● 16 bit	● Not reported	
		SIGNAL PREPROCESING		
PREPROCESSING	● Allows for manual visual inspection	● Allows for manual visual inspection	● *Recommends* manual visual inspection *prior* to using program	
SAMPLING FREQUENCY	● Sampled at various rates resulting in QRS timing at different resolutions; 1024 samples/300 s	● 1000 Hz	● Beat-to-beat RR intervals	● Optimal range is 250-500 Hz or higher (Task Force, 1996)
R-PEAK DETECTION	● Based on template matching; use a special cross-correlation for upcoming signal with all templates already formed	● Provides low-pass or high-pass filter raw ECG data at a manually adjustable cut-off frequency	● In-house algorithm similar to Pan-Tompkins algorithm	● Necessary to use well-tested algorithm (template, correlation method, derivative plus threshold)
RR INTERVAL FILTERING & INTERPOLATION	● Automated and manual filtering available	● Automated and manual filtering available	● Automated filtering only ● Smoothness prior to detrending	● LF cutoff = 0.05 Hz ● HF cutoff = 150 Hz

	MARS (GE MARQUETTE)	MINDWARE	KUBIOS HRV	TASK FORCE
DETREDNING ALGORITHM OR AUTOREGRESSIVE SPECTRUM MODEL	● A linear trend is fit to FFT input samples of a 600 s window ● Middle 5 min detrended	● Linearly detrended	● AR model order: 16 ● None, 1st- 3rdorder ● Smooth priors	
RESAMPLING OR INTERPOLATION RATE	● Spline model to interpolate to 1024 samples evenly spaced data for spectral analysis	● Resampled at frequency based on 200 bpm/60 x 10 or 33.33 Hz	● 4 Hz (default; adjustable)	● Spectral analysis should use at least 512 but preferably 1024 samples for 5 min recordings
INTERPOLATION METHOD	● Cubic spline interpolation; used for discrete event series (DES)	● Cubic spline interpolation	● Cubic spline interpolation	● Regularly sampled interpolation of DES with (non) parametric methods
WINDOW WIDTH	● 300 s	● Not reported	● 256 s (default; adjustable)	
WINDOW OVERLAP	● Spectral generated every minute; 4 min or 80%	● Not reported	● 50% re-sampled data (adjustable)	
WINDOWING	● Hanning ● Spectral coefficients are scaled to properly account for the attenuation of signal energy due to window	● Hamming	● Hanning ● Points in frequency-domain: 256 points/Hz (adjustable)	● Hanning & Hamming
SPECTRUM METHOD	● Periodogram	● Standard power spectrum	● Welch's periodogram method	
		DATA CLEANING		
ARTIFACT DETECTION & HANDLING	● Manual handling artifacts ● Uses full suite of GE Marquette® algorithms	● Manual handling artifacts ● Dual ECG artifact detection algorithms, MAD/MED and IBI check (Berntson et al., 1990)	●Artifact Correction Options: (None, very low, low, medium, strong, very strong)	● Proper interpolation on preceding or successive beats on HRV signal or on autocorrelation function

	MARS (GE MARQUETTE)	MINDWARE	KUBIOS HRV	TASK FORCE
	AUTOMATIC ANALYSIS HRV INDICES			
TIME-DOMAIN VARIABLES	● Mean RR (ms) ● SDNN (ms) ● SDANN (ms) ● SDNN index(ms) ● rMSSD (ms) ● NN50 (counts) ● pNN50 (%)	● Mean RR (ms) ● SDNN (ms) ● Mean HR ● rMSSD (ms) ● NN50 (counts) ● pNN50 (%)	● Mean RR (ms) ● SDNN (ms) ● SDANN (ms) ● Mean HR* (1/ms) ● SD HR (1/ms) ● rMSSD (ms) ● NN50 (counts) ● pNN50 (%)	● SDNN (ms) ● SDANN (ms) ● SDNN index(ms) ● rMSSD (ms) ● NN50 (counts) ● pNN50 (%)
	*Calculated for each epoch & averaged across entire recording period	*Calculated for each epoch & averaged across entire recording period	*Calculated for entire recording period	
FREQUENCY BANDS	● VLF (0.0033-0.04 Hz) ● LF (0.0400-0.15 Hz) ● HF (0.1500-0.4 Hz)	● VLF (0.0030-0.0400 Hz) ● LF (0.0400-0.1500 Hz) ● HF (0.1500-0.4000 Hz)	● VLF (0.00-0.04 Hz) ● LF (0.04-0.15 Hz) ● HF (0.15-0.4 Hz)	● VLF (0.00-0.04 Hz) ● LF (0.04-0.15 Hz) ● HF (0.15-0.4 Hz)
UNITS	● Hz, ms^2	● Hz, ms^2	● Hz, ms^2, %, n.u.	● Hz, ms^2, %, n.u.
EXTRA OUTPUT DATA	● # of Rs found (complex QRS) ● Ventricular beats (<1%) ● Supraventricular beats (<1%)	● # of Rs found ● RSA ● First ECG R time	● Geometric parameters: RR triangular index, TINN ● Poincare Plot: SD1 & 2 ● Recurrence plot analysis, Correlation dimension etc.	
EXPORT OPTIONS	● Adobe Acrobat PDF	● ASCII	● Adobe Acrobat PDF, Matlab MAT-file, ASCII	

Table 2

Measurement Fidelity for Heart Rate Variability Parameters across Software Programs

	MARS vs. MindWare		MARS vs. Kubios		MindWare vs. Kubios	
	ICC (95%CI)	*r*	*ICC (95%CI)*	*r*	*ICC (95%CI)*	*r*
Mean RR (ms)	0.98 (0.93, 0.99)	0.96**	0.98 (0.94, 0.99)	0.96**	1.00 (0.99, 1.00)	1.00**
Time-domain						
SDNN (ms)	0.93 (0.80, 0.98)	0.94**	0.97 (0.93, 0.99)	0.97**	0.99 (0.96, 1.00)	0.99**
SDANN(ms)	0.90 (0.73, 0.97)	0.86**	0.90 (0.71, 0.96)	0.82**	0.98 (0.95, 0.99)	0.97**
SDNNi (ms)	0.93 (0.80, 0.98)	0.94**	0.98 (0.95, 0.99)	0.97**	0.98 (0.94, 0.99)	0.99**
rMSSD (ms)	0.62 (-0.07, 0.86)	0.87**	0.83 (0.52, 0.94)	0.94**	0.96 (0.88, 0.99)	0.97**
pNN50 (%)	0.96 (0.89, 0.99)	0.98**	0.97 (0.91, 0.99)	0.98**	1.00 (0.99, 1.00)	1.00**
Frequency-domain						
VLF (ms^2)	0.77 (0.34, 0.92)	0.95**	-0.49 (-3.15, 0.48)	0.76**	0.29 (-0.99, 0.75)	0.79**
LF (ms^2)	0.82 (0.50, 0.94)	0.90**	0.91 (0.74, 0.97)	0.96**	0.98 (0.93, 0.99)	0.97**
HF (ms^2)	0.87 (0.64, 0.95)	0.96**	0.95 (0.87, 0.98)	0.97**	0.92 (0.79, 0.97)	0.95**
LF:HF ratio	0.93 (0.79, 0.97)	0.90**	0.96 (0.90, 0.99)	0.95**	0.89 (0.71, 0.96)	0.94**

Note. ICC= Intraclass Correlation Coefficient; *r* = Pearson Correlation Coefficient; CI = Confidence Interval; Mean RR = Mean beat-to-beat intervals; SDNN = Standard deviation of all RR intervals; SDANN = Standard deviation of the averages of RR intervals in all 5 min segments of the entire recording; SDNNi = Mean of the standard deviations of all RR intervals for all 5 min segments of the entire recording; rMSSD = Square root of the mean of the squares of differences between adjacent RR intervals; pNN50 = Proportion derived by dividing the number of interval differences of successive RR intervals greater than 50 ms by the total number of RR intervals; VLF = Very Low Frequency; LF = Low Frequency; HF = High Frequency. $^{*}p < .05$, $^{**}p < .01$

Table 3

Means and Standard Deviations of Heart Rate Variability Parameters across Software Programs

	MARS	MindWare	Kubios	
	M (SD)	*M (SD)*	*M (SD)*	*F*
Mean RR (ms)	700.56 (51.88)	705.32 (48.57)	700.03 (48.87)	0.06
Time-domain				
SDNN (ms)	84.38 (21.81)	93.45 (23.59)	89.13 (22.01)	0.65
SDANN (ms)	40.50 (12.36)	43.69 (15.52)	41.94 (15.05)	0.20
SDNNi (ms)	73.00 (20.87)	81.51 (22.18)	76.41 (20.19)	0.66
rMSSD (ms)	50.56 (14.65)	70.08 (25.86)	62.18 (22.96)	3.28
pNN50 (%)	27.89 (12.79)	32.29 (14.20)	31.72 (13.91)	0.49
Frequency-domain				
VLF (ms^2)	1220.98 (586.32)	1822.60 (936.91)	3715.50 (1880.56)	17.10^{**abc}
LF (ms^2)	1280.83 (873.85)	1934.72 (1069.67)	1764.50 (927.43)	2.00
HF (ms^2)	996.22 (773.37)	1524.21 (1082.78)	1267.75 (737.91)	1.45
LF:HF ratio	1.49 (0.66)	1.70 (0.68)	1.35 (0.60)	1.15

Note. M = Mean; SD = Standard Deviation; F = F test-statistic from omnibus ANOVA; Superscript denotes follow-up pairwise comparison: [a]MARS vs. MindWare. [b]MARS vs. Kubios. [c]MindWare vs. Kubios; Mean RR = Mean beat-to-beat intervals; SDNN = Standard deviation of all RR intervals; SDANN = Standard deviation of the averages of RR intervals in all 5 min segments of the entire recording; SDNNi = Mean of the standard deviations of all RR intervals for all 5 min segments of the entire recording; rMSSD = Square root of the mean of the squares of differences between adjacent RR intervals; pNN50 = Proportion derived by dividing the number of interval differences of successive RR intervals greater than 50 ms by the total number of RR intervals; VLF = Very Low Frequency; LF = Low Frequency; HF = High Frequency. $^{**}p < .01$

TRANSITION TO MANUSCRIPT 2

The purpose of manuscript 1 was to investigate the correspondence across three common signal processing software programs used to derive HRV indices. Using triplicate ECG data derived from identical data acquisition hardware, the comparability of HRV indices for time- and frequency-domain variables was tested. Additionally, this manuscript highlighted the importance of reporting the various signal processing techniques and specifications.

Manuscript 1 was an innovative investigation demonstrating that rigorous user-defined technical specifications for nuanced HRV processing details yields strong to excellent correspondence for most HRV indices, across signal-processing software programs. These findings have important implications for the field and suggest with comparable methodological, cleaning, and technical specifications, HRV derived from different software programs can be compared.

Although HRV is used in the pediatric literature, no referent or normative HRV values exist. This limits the ability to make comparisons across child studies, even if different software programs can yield comparable HRV indices. To achieve my overarching goal of testing whether sympathovagal balance mediates the association between sleep and obesity, it would be prudent to first establish HRV reference values with this population. Referent HRV values would facilitate comparison with previously reported values and the capacity to synthesize the existing literature. Further, standard covariates, particularly those that are developmentally relevant, have not been systematically evaluated in children. Taken together, these gaps posed challenges in the interpretation of HRV in children, which evolved into my second methodological

endeavour presented in manuscript 2. The two objectives of manuscript 2 were: (1) to establish normative HRV reference values from a large population-based sample of children, and (2) to test potential developmentally relevant variables as possible standard covariates for HRV in children.

MANUSCRIPT 2:

Short-Term Heart Rate Variability in a Population-Based Sample of 10-Year-Old Children

Jarrin, D. C., McGrath, J. J., Poirier, P., Séguin, J. R., Séguin, L., Tremblay, R. E., &, Paradis, G. (Submitted.)

Abstract

Heart rate variability (HRV) is a valuable quantitative marker that provides information on the flexibility and balance of cardiac sympathetic and parasympathetic activation. Despite its widespread use within pediatric populations, normative values to serve as a reference to synthesize existing findings and for comparison purposes are lacking in the field. In addition, the unique variance of developmentally relevant covariates (e.g., puberty) on HRV has not been systematically evaluated in children. The objectives of the present study were twofold. The first objective was to provide time- and frequency-domain HRV values for a large, population-based sample of 10-year-old children. The second objective was to evaluate developmentally relevant variables as possible covariates of HRV. Participants included 1052 healthy children aged 9 to 11 years who participated in the Québec Longitudinal Study of Child Development cohorts. To promote comparative studies and facilitate synthesis of findings across studies within pediatric populations, normative reference values of HRV variables are presented in the current study. Results revealed several developmentally relevant covariates (e.g., heart rate, pubertal status, blood pressure, sex, sleep measures, physical activity) that are pertinent when analyzing HRV in children. Researchers should be prudent in considering these covariates, which differ depending on the HRV parameter of interest, given the research question. Altogether, these data have potential implications to advance the field, as these referent values may facilitate comparison and synthesis of previously reported HRV data among pediatric studies.

Introduction

The naturally occurring oscillation in heart rate is referred to as heart rate variability (HRV). HRV is a physiological measure directly reflecting the modulating influences and balance of the cardiac autonomic nervous system. HRV is one of the most widely used methods for measuring cardiac autonomic function as it is a sensitive, reproducible, and non-invasive measure that is easily derived from continuous ECG recordings (Berntson, Cacioppo, & Quigley, 1991; Berntson, Cacioppo, & Quigley, 1993; Bonnet, 2012; Cacioppo, 1994; Task Force, 1996). Reduced HRV reflects the inability or attenuation of the autonomic regulatory capacity to support flexible adjustments in response to the environment, while heightened HRV reflects the optimal ability of the autonomic nervous system to respond adaptively (McMillan, 2002; Thayer & Sternberg, 2006). Autonomic imbalance is associated with assorted cardiac (e.g., Liao, Carnethon, Evans, Cascio, & Heiss, 2002) and non-cardiac pathologies (e.g., obesity, anxiety: Kaufman, Kaiser, Steinberger, & Dengel, 2007; Martini et al., 2001; Sharma, Balhara, Sagar, Deepak, & Mehta, 2011). In children, researchers have examined HRV to identify early manifestations of varying health conditions (e.g., sub-clinical seizures; Brotherstone & McLellan, 2012), to quantify the influence of disease progression (e.g., obesity; Kaufman et al., 2007), to understand mechanisms of developmental psychopathology (e.g., anxiety; Mezzacappa et al, 1997), and to assess the efficacy of therapeutic interventions (e.g., physical training; Nagai, Matsumoto, Kita, & Moritani, 2003; Nagai & Moritani, 2004;).

Despite the increased use of HRV in the pediatric literature, the lack of established HRV normative values make it difficult to synthesize results and draw

meaningful comparisons across studies in the field. Few studies report descriptive HRV values within child populations that could be used for comparative or reference purposes (c.f., Finley, Nugent, & Hellenbrand, 1987; Goto et al., 1997; Massin & von Bernuth, 1997; Umetani, Singer, McCraty, & Atkinson, 1998). Multiple methodological and measurement differences exist across the extant literature. Sample sizes are often extremely small (e.g., n = < 10) or combine youth across broad age spans (e.g., 2 to 16 years; Kwok et al., 2011). "Normal" HRV values are typically reported based on children recruited as "healthy controls" (c.f., Chen, 2012; Rydberg, Rask, Hörnsten, & Teien, 2004; Yang et al., 2001) or from highly specific samples (e.g., swimmers; Vinet, Beck, Nottin, & Obert, 2005) or even clinical samples (e.g., Białłkowski et al., 2003). Measurement differences include the use of varying duration of electrocardiogram (ECG) recordings (e.g., 2 min to 24 hrs), use of laboratory tasks or conditions (e.g., tasks vs. normal routine), as well as inconsistent and injudicious reporting of HRV values (e.g., beats/min^2; Finley et al., 1987). Further, developmentally relevant covariates in childhood (e.g., puberty) are often not considered. Altogether, the state of knowledge regarding "normal" HRV values in children is rather haphazard. In the absence of established normative values and standard covariates, as well as a lack of a comprehensive methodological framework in which to investigate normative values among children, progress in the field is limited.

To further illustrate the disarray of the current state of HRV within pediatric populations, the existing literature to date was reviewed to systematically organize and better estimate plausible reference values for short- and long-term HRV values in healthy children. An overview of cross-sectional studies reporting HRV values, with a minimal

sample size of 10 and a mean age of 10 years (range 6 to 18 years), is outlined in Table 1 (short-term recording of 3 to 60 min) and Table 2 (long-term recording greater than 20 hours). Normal values were typically reported for participants recruited as age- and sex-matched controls and deemed healthy based on parental-report, medical history, or exclusion criteria (e.g., no cardiac pathology).

HRV values reported in the extant literature were computed based on traditional time-and frequency domain analyses. Time-domain variables include standard deviation of all beat-to-beat (RR) intervals (SDNN), standard deviation of the averages of RR intervals in all 5 min segments of the entire recording (SDANN), mean of the standard deviations of all RR intervals for all 5 min segments of the entire recording (SDNNi), square root of the mean of the squares of differences between adjacent RR intervals (rMSSD), and the proportion derived by dividing the number of interval differences of successive RR intervals greater than 50 ms by the total number of RR intervals (pNN50). Frequency-domain variables include very low frequency (VLF), low frequency (LF), high frequency (HF), and LF:HF ratio.

While some studies were very thorough in assessing health status (e.g., clinical examination by a physician, urine analysis, blood tests, cardiac ultrasound, performing a resting ECG prior to testing; c.f., Henje Blom et al., 2009; Kaufamn et al., 2007; Martini et al., 2001; Silveti, Drago, & Ragonese, 2001), others used less rigorous, albeit common, methods of assessing health status (i.e., parent-report; Nagai & Moritani, 2004; Nagai et al., 2003). More concerning was that some studies provided no information regarding health status (e.g., Dundaroz et al., 2001; Fujuiwara et al., 2001) or included participants with minor cardiac conditions (i.e., innocent murmurs; Karacan, Ceviz, & Olgun, 2011).

Relatedly, while some publications had stringent exclusion criteria (e.g., free of major cardiovascular or metabolic diseases, thyroid dysfunction, unexplained syncopal episodes, neurological deficit, obstructive sleep apnea; Henje Blom et al., 2009; Kaufamn et al., 2007), others provided insufficient details (c.f., Fujiwara et al., 2001; Dundaroz et al., 2001). The majority of studies excluded use of medications known to interfere with cardiovascular function and restricted consumption of caffeinated beverages as well as moderate to strenuous physical activity for 24 hours prior to the ECG recording.

For the derivation of HRV indices, some publications failed to adhere to the Task Force (1996) recommendations for spectral analyses. Frequency bandwidths for LF and HF parameters were inaccurate in a few studies (c.f., Finley & Nugent, 1995; Fujiwara et al., 2001; Nagai & Moritani, 2004; Nagai et al., 2003), and in other studies, no information on frequency bandwidths was provided (c.f., Chen, Lee, Chiu, & Jeng, 2008; Karacan et al., 2011; Winsley, Armstrong, Bywater, & Fawkner, 2003). In one case, VLF was derived from a 4 min recording (McCarty, Atkinson, & Tomasino, 1999), which is not recommended because the physiological meaning of VLF is dubious under such short recording lengths (Task Force, 1996).

To further add to the confusion in assessing normative HRV values, some studies only present HRV values graphically (c.f., Finely et al., 1987; Tonhajzerova et al., 2010; Massin, Maeyns, Withofs, Ravet, & Gérard, 2000), while other studies report HRV values in transformed (e.g., log-transformed), absolute (e.g., ms^2), and/or normalized (e.g., %) units. Additionally, other studies report HRV values in units that differ from standard units (e.g., $beats/min^{2,}$ Hz; Finley & Nugent, 1995; Heragu & Scott, 1999; Yeragani, Rao, Pohl, Jampala, & Balon, 2001). This is a particularly salient issue for

frequency-domain parameters, which evidenced extreme ranges in Tables 1 and 2. For example, LF ranged from 74.1 to 2859 in absolute (ms^2), 3.57 to 7.40 in log-transformed (ln), and 19.8 to 54.9 in normalized units (n.u., %). Time-domain variables were largely consistent, with the exception of a few studies that reported transformed values or used seconds rather than the standard millisecond unit (c.f., Henje-Blom et al., 2009; Kaufamn et al., 2007; Kwok et al., 2011; Winsley et al., 2003). As such, the current state of the literature has several methodological weaknesses and inconsistencies that hinder the comparison of HRV values across pediatric studies.

The selection and use of covariates was also disparate across studies. Few studies reported nor statistically controlled for pubertal status (Chen, Chiu, Lee, Sheen, & Jeng, 2012; Faulkner, Hathaway, & Tolley, 2003; Gamelin et al., 2009), sleeping habits, habitual physical activity, blood pressure, or resting heart rate (e.g., Henje Blom et al., 2009; Kwok et al., 2011; Wang, Thayer, Treiber, & Snieder, 2005). These developmentally relevant covariates are particularly important when considering a comprehensive methodological framework to investigate normative HRV values during childhood; their absence in the existing literature is poignant.

Childhood is a critical developmental period during the lifecourse when there are profound hormonal changes related to puberty (Rogol, Clark, & Roemmich, 2000), significant changes in sleep habits (i.e., later bed time, shorter sleep duration, weekend oversleeping) due to contextual restraints (e.g., early school start times, extracurricular activities) and physiological influence of the suprachiasmatic nucleus (i.e., delay of circadian phase; Carskadon, Acebo, Richardson, Tate, & Seifer et al., 1997; Laberge et al., 2001), and variations in physical activity (Sallis, Buono, Roby, Micale, & Nelson,

1993). There are significant age-related changes in blood pressure and heart rate, such that systolic blood pressure (SBP) and diastolic blood pressure (DBP) increases with age (Soergel et al., 1999), while heart rate decreases with age (Fleming et al., 2011; Salameh et al., 2008; Wallis, Healy, Undy, & Maconochie, 2005). Collectively, these covariates have potential developmental implications on HRV parameters; yet, they are inconsistently considered in pediatric studies.

Based on limited findings, younger age (Lenard, Studinger, Mersich, Kocsis, & Kollai, 2004; Silvetti, Drago, & Ragonese, 2001), greater physical activity (Chen et al., 2012; Nagai & Moritani, 2004; Nagai et al., 2003;), and consolidated sleep (Kwok et al., 2011; Liao et al., 2010) are associated with a predominance of parasympathetic activity and reduced sympathetic modulation. Of the few studies examining puberty, there are inconsistent results on the association between pubertal maturation and HRV (Chen et al., 2012; Faulkner et al., 2003), which may be attributable to differences in the age range of each sample. Thus, an existing gap in the literature concerns referent HRV values during childhood and identification of developmentally relevant covariates.

In sharp contrast to the pediatric literature, numerous adult population-based studies have reported normative HRV values, and have not only considered important covariates, but have also looked at the role of *multiple* covariates (c.f., Jensen-Urstad, et al., 1997; Nunan, Sandercock, & Brodie, 2010; Stein, Kleiger, & Rottman, 1997; Sinnreich, Kark, Friedlander, Saporznikov, & Luria, 1998; Task Force, 1996; Tsuji et al., 1996). Sex, age, and heart rate are consistently the most robust covariates (Stein, Kleiger, & Rottman, 1997; Tsuji et al., 1996). Other covariates known to influence adult HRV include adiposity (Poliakova et al., 2012), total processing time (i.e., ECG data

analyzed), blood pressure, medication use, and physical activity (c.f., Berntson et al., 1997; Malpas & Purdie, 1990; Shannon, Carley, & Benson, 1987; Task Force, 1996). Despite the robust literature among adults regarding reference HRV values and standard covariates, unique developmental changes and milestones limit these values from being extrapolated to children.

Normative HRV values during childhood remain to be established. Further, although many studies control for age, sex, and medication use, to the best of our knowledge, no studies have been performed with the aim of explicitly assessing developmentally relevant covariates, both singularly and collectively, on HRV values in children. Current discrepancies in the existing pediatric literature exacerbate the confusion and difficulty in the interpretation of HRV across studies, and ultimately, hinder the synthesis of findings and advancement in the field. The objectives of the present study were twofold. The first objective was to provide time- and frequency-domain HRV values for a large, population-based sample of 10-year-old children. The second objective was to evaluate developmentally relevant variables as possible covariates of HRV.

Method

Participants and Procedure

Participants included 1052 children who participated in the population-based Québec Longitudinal Study of Child Development (QLSCD). The QLSCD is an ongoing study conducted by the Québec Institute of Statistics (Jetté, 2002; Jetté, Desrosiers, & Tremblay, 1997; Jetté & Desgroseillers, 2000). The original cohort was selected from the Ministere de la Santé et des Services Sociaux's master birth registry using a multistage cluster (by region and municipality) random sampling strategy that was representative of

singleton births born from 1996 to 1998. At the time of original sampling, exclusion criteria included serious medical pathology, infants born before 24 or after 42 weeks gestation (0.1%) and those with unknown gestational age (1.3%), families living in Aboriginal territories or remote regions of Québec, Canada, and parents who did not understand French or English. The original QLSCD sample represented 97.8% of the target population and was approved by the ethics review boards of the Institut de la statistique du Québec, the Centre Hospitalier Universitaire (CHU) Sainte-Justine, the Louis-Hippolyte Lafontaine Hospital, and the Faculty of Medicine of Université de Montréal. (Further information on survey methodology and data sources can be retrieved online http://www.iamillbe.stat.gouv.qc.ca/default_an.htm.)

The present analyses are based on the cross-sectional detailed cardiovascular health screening that was conducted between 2006 and 2008 as part of the QLSCD yearly follow-up assessment at age 10 years. Parents and children were invited to participate and provided informed consent and assent, respectively, for the cardiovascular health assessment. During the scheduled visit, parents and children completed questionnaires. Children had their anthropometric measures taken by a registered nurse and had their ECG signal continuously recorded throughout the standardized visit. Children also completed a questionnaire on other developmental aspects of this population survey that were not related to this study.

Measures

Heart Rate Variability. While participants were seated quietly, continuous raw ECG data were acquired, digitized (128 Hz), and recorded using the 8500 Marquette MARS Holter monitor (GE Marquette Medical Systems, Milwaukee, Wisconsin, USA).

To reduce violations of stationarity, the procedure was kept consistent for all participants (Berntson et al., 1997). The Holter monitor incorporated a quartz-derived, binary time channel that was automatically zeroed at the start of the recording. A modified Lead II electrode configuration with three disposable, pregelled snap silver chloride electrodes were used to acquire an ECG signal. Electrode resistance was kept low (<10 kO) by cleaning the skin with a rubbing alcohol swab. The active electrode [and its derivative (dZ)] was placed on the right clavicle next to the sternum over the first rib between the two collarbones. The second electrode was placed on the left mid-clavicular line at the apex of the heart over the ninth rib. The ground electrode was placed at the lowest possible right rib cage on the abdomen. Additional dZ electrodes were placed over the right fourth intercostal space at the sternal edge, the fifth intercoastal space at the left axillary line, and on the sixth rib in the mid-clavicular line.

ECG Signal Processing. ECG data were uploaded on the MARS® Holter Analysis Workstation (GE Marquette Medical Systems, Milwaukee, Wisconsin, USA), where data was formatted for viewing, editing, and ECG interpretation and analysis. Beat-by-beat visual inspection of the shape, trend, and length of each QRS complex were identified based on standard Marquette algorithms for QRS labeling and further verified by visual inspection from a qualified trained professional. ECG data were sampled at 1024 samples/300 s and RR intervals were automatically filtered. The removal of artifacts was based on a 20% change from the previous signal as a criterion (Kleiger, Miller, Bigger, & Moss, 1987). In cases where artifacts and excluded RR intervals were automatically filtered and identified as unreadable signals, the remaining acceptable beats were used to replace the data points via cubic spline interpolation method. At least four

acceptable R-peaks were needed in order for spline interpolation to identify the continuous function between two middle R-peaks.

Next, input samples of 10 min were linearly detrended, mean-centered, and tapered using a Hanning window, and processed by Fast Fourier Transform (FFT) periodogram spectrum method. Frequency-domain variables included VLF (0.0033-0.04 Hz), LF (0.04-0.15 Hz), HF (0.15-0.4 Hz), as well as LF:HF ratio and were calculated and expressed in absolute units. Time-domain variables included SDNN, SDANN, SDNNi, rMSSD, and pNN50.

Blood Pressure. An appropriate-sized occlusion cuff was attached to the medial surface of the right arm over the brachial artery using an oscillometric instrument (BpTRU, model BPM-100, VSM MedTech Ltd, Vancouver, Canada) according to standardized procedures (Webber et al., 1995). Prior to data collection, the blood pressure units were calibrated with a mercury sphygmomanometer to ensure precision. Five blood pressure and heart rate readings were taken at 1 min intervals while the participant was seated. The mean of all readings were calculated for SBP, DBP, and heart rate.

Anthropometrics. Using a standard measuring tape, waist circumference was measured at the narrowest part of the abdomen, midway between the lowest rib and the iliac crest; hip circumference was measured at the widest part of the body over the buttocks. Height was measured using a standard measuring tape to the nearest 0.1 cm with shoes off. Weight was measured with a calibrated spring scale to the nearest 0.1 kg while the participant was dressed in light clothing. Height and weight were measured in duplicate; if the difference between the first two measurements exceeded 0.5 cm for

height and 0.2 kg for weight, a third measurement was taken and the average of the two closest measurement was used in the analysis. Height and weight measurements were used to calculate body mass index (BMI). BMI [weight in kg/(height in meters^2)] was converted to age-and sex-specific BMI percentiles Z-scores determined using the growth charts published by the U.S. Centers for Disease Control and Prevention (Ogden, Flegal, & Carroll, 2002).

Puberty. Using a validated self-report measure of puberty (Growing and Changing Questionnaire; Golding et al., 2001), two stages of pubertal development were assessed: gonardarche (breast and genital development) and adrenarche (pubic hair). Youth indicated their pubertal stage based on sex-specific illustrations corresponding to Tanner stages I-V of prepubertal to complete sexual maturity. Females were also asked if they had started menstruating (response options: yes or no). Although physician assessment of pubertal development is considered the gold standard, self-report has demonstrated good reliability and validity among youth (r = 0.77 to 0.91; Morris & Udry, 1980; Netherton, Goodyer, Tamplin, & Herbert, 2004).

Sleep. Parents reported on their child's typical bed- and wake-time on school and weekend nights. Average, school, and weekend night sleep durations were calculated as the difference between bed- and wake-times. Parents reported the sleep onset latency (amount of time it takes for their child to fall asleep in min) and whether their child woke up after sleep onset (response: yes or no).

Physical Activity, Medication, and Caffeine Intake. Children reported on their habitual physical activity in the past week (range: almost every day to almost never). Parents reported on their child's medication use in the past two weeks. Medications were

categorized by a cardiologist into general and prescription medications with and without known cardiovascular effects. Parents also reported whether their child exercised vigorously, consumed any caffeine products (e.g., chocolate, energy drinks) and or medication (response options: yes or no) on the day of the ECG recording.

Statistical Analyses

All data were entered and double-checked by the senior data coordinator and analyzed with IBM SPSS Statistics 20 software (SPSS, Inc., Chicago, IL). The distributions of frequency-domain variables (VLF, LF, HF) were highly skewed and thus, natural log-transformed. To yield normative values, mean and standard deviations were estimated for each HRV parameter stratified by heart rate reference ranges based on age (Wallis et al., 2005) at the 5^{th}, 25^{th}, 50^{th}, 85^{th}, and 95^{th} percentiles. Using multiple linear regression, HRV values were estimated while controlling for age, sex, and heart rate. Second, simple linear regression models were used to test the unique variance explained by each covariate for every HRV parameter (SDNN, SDANN, SDNNi, rMSSD, pNN50, VLF, LF, HF, LF:HF ratio). Finally, for each HRV parameter, post-hoc stepwise regression models were used to identify the most salient covariates when all were entered simultaneously.

Results

Of the original 1052 participants completing the cardiovascular assessment at the scheduled QLSCD follow-up visit, 12 were excluded due to missing ECG recordings and four were excluded due to insufficient recording duration (less than 30 min), yielding a final sample of 1036. Participant demographics are presented in Table 3. All children were free of cardiovascular pathology (e.g., bradycardia, fibrillation, premature

contraction) based on review of the ECG recordings reviewed by a board-certified cardiologist. The average length of the ECG recordings was 69 min (*SD* = 21). All ECG recordings were inspected manually by a cardiovascular technician to review peak detection and to identify and remove artifacts; manual inspection and editing took approximately 12 min per recording. Recordings were found to be of excellent quality; over 97% of data were analyzable, artifact time did not exceed 8 min (0.09%), and no recordings were found to exceed 20% noise or ectopic beats. ECG recordings typically began in the mid-morning (10:41, *SD* = 3:13) and the majority of children refrained from strenuous physical activity (96.4%), caffeine (87.6%), and medication use (82%) for the prior 24 hours.

Children's health status was "very good" to "excellent" (95.3%) based on parental-report. More than half were female (53.6%), Caucasian (87%), of normal weight status (73% BMI 5-85th percentile), and physically active 5 to 6 days/week (37%). On average, children were 10.21 years (*SD* = 0.29) and identified being in the early stages of gonardarche (*M* = 1.85, *SD* = 0.64) and adrenarche (*M* = 1.73, *SD* = 0.69); only 1.8% of females (*n* = 3) reported having started their menses. Parents reported their child slept for an average of 612 min (*SD* = 32) and 616 min (*SD* = 48) on school and weekend nights, respectively, and took 18 min (*SD* = 16) to fall asleep. On average, the child's bed-time was at 20:31 on school nights and 21:36 on weekend nights.

Children's blood pressure and heart rate fell within normal limits (SBP = 97 mmHg, *SD* = 10.06; DBP = 62 mmHg, *SD* = 8.82; HR = 80.17 beats/min, *SD* = 10.17). Approximately 60% of participants reported not taking any medication in the prior two weeks. Of those reporting medication use (*n* = 419), 0.7% were taking general

medication without cardiovascular effects (e.g., Lactaid), 28% were taking general medication with cardiovascular effects (e.g., Claritin), 2% reported taking prescription medication without cardiovascular effects (e.g., antibiotics), and 9% reported taking prescription medication with cardiovascular effects (e.g., Ritalin).

Mean values and standard deviations for heart rate, RR intervals, as well as time- and frequency-domain HRV variables (absolute and log-transformed units) are presented in Table 3. Overall, boys showed significantly greater HRV values for all time-and frequency-domain variables, except LF:HF ratio. Normative HRV values for the 5^{th}, 25^{th}, 50^{th}, 85^{th} and 95^{th} percentiles stratified by age, sex, and heart rate are presented in Table 4. Due to the consistent age-related changes of heart rate (Fleming et al., 2011; Wallis et al., 2005), HRV values accounting for age, sex, and heart rate were estimated using linear regression equations.

Regression analyses revealed several significant covariates for HRV parameters. Across time-domain variables, greater SDNN, SDANN, and SDNNi values were associated with male sex, reduced SBP, DBP, and HR, earlier start times, earlier bed-times, and longer sleep duration (Table 5). Greater SDNN and SDANN values were also associated with longer processing time. Higher rMSSD and pNN50 values were associated with older age, male sex, reduced SBP, DBP, and heart rate, as well as earlier pubertal stage.

For frequency-domain variables, higher VLF, LF, and HF values were associated with male sex, lower SBP, DBP, and heart rate, earlier start times, earlier bed-times on weekends, and longer sleep duration (Table 5). Greater VLF and LF were associated with longer processing time and earlier bed-times on school nights. Greater VLF also

was associated with reduced waist circumference. Greater HF was associated with earlier pubertal stages and fewer nocturnal awakenings.

Greater LF:HF ratio was associated with younger age, female sex, increased heart rate, earlier start times, longer processing time, advanced pubertal stage, more nocturnal awakenings, and less physical activity. Altogether, heart rate accounted for the greatest amount of variance in HRV values (R^2_{avg} = 39%), followed by blood pressure (R^2_{avg} = 5.33%), start time of ECG recordings (R^2_{avg} = 3.48%), processing time (R^2_{avg} = 1.61%), and pubertal status (gonardarche = 0.97%; adrenarche = 0.80%).

Post-hoc analyses revealed patterns of covariates unique to each HRV when entered simultaneously. Among the time-domain variables, for SDNN and SDNNi, heart rate (β_{avg} = -0.65), ECG recording start time (β_{avg} = -0.19), and DBP (β_{avg} = -0.11) were significant covariates. For SDANN, heart rate (β = -0.43) and processing time (β = 0.26) were significant covariates. For rMSSD, heart rate (β = -0.72), male sex (β = 0.10), DBP (β = -0.10), gonardarche pubertal status (β = -0.09), and ECG recording start times (β = -0.09) were significant covariates. For pNN50, heart rate (β = -0.73), and gonardarche pubertal status (β = -0.10) were significant covariates.

Among the frequency-domain variables, for VLF, heart rate (β = -0.64), ECG recording start time (β = -0.27), and DBP (β = -0.10) were significant covariates. For LF, heart rate (β = -0.63) and ECG recording start time (β = -0.14) were significant covariates. Only heart rate remained a significant covariate for HF (β = -0.65). Finally, for LF:HF ratio, heart rate (β=0.31) and gonardarche pubertal status (β = -0.14) were significant covariates. Overall, the most consistent covariate that accounted for the most

variance in *all* HRV parameters was heart rate (range from 8.4% to 56.1% of the variance).

Discussion

HRV is a valuable quantitative marker that provides information on the flexibility and balance of cardiac sympathetic and parasympathetic activation. Despite its widespread use within pediatric populations, normative values to serve as a reference to facilitate comparisons across studies and synthesize existing findings are lacking in the field. In addition, standard covariates, particularly those that are developmentally relevant, have not been systematically evaluated in children. Taken together, these limitations pose challenges in the interpretation of HRV in children. The first objective was to provide time- and frequency-domain HRV values for a large, population-based sample of 10-year-old children. The second objective was to comprehensively assess the influence of several covariates on commonly used HRV parameters. To the best of our knowledge, this is the first study to provide normative HRV values for children and to evaluate *multiple* developmentally-relevant covariates.

Normative Heart Rate Variability Values

In comparison to the reviewed studies that have previously reported HRV values for children similarly aged (see Table 1 & 2), some differences were noted. Compared to studies using comparable length short-term recordings (i.e., 2 to 60 min), larger SDNN, LF, and LF:HF ratio values and smaller rMSSD and pNN50 values were observed in the population-based sample. Previous studies have not reported values for SDANN or SDNNi. The findings were largely consistent for SDNN, rMSSD, and HF. In addition, many values reported in previous research were within the 5^{th} and 95^{th} percentiles values

presented in Table 4. These discrepancies may be attributable to methodological factors such as, small sample sizes (Winsley et al., 2003), differing male to female ratios (Faulkner et al., 2003), posture (e.g., seated vs. supine; Chen et al., 2012; Sharma et al., 2012), differing data reduction techniques (Karacan et al., 2011), as well as inconsistencies in units and frequency bandwidths used across studies (Finley & Nugent, 1995; Finley et al., 1987).

Covariates

Heart Rate and Blood Pressure. Overall, consistent covariates of HRV in children were heart rate, SBP, and DBP. Consistent with the adult literature, reduced heart rate and blood pressure were significantly associated with greater time-and frequency-domain HRV variables (Castiglioni, Di Rienzo, Veicsteinas, Parati, & Merati, 2006; Palatini & Julius, 2009; Schroeder et al., 2003; Tsuji et al., 1996). In a study among adolescents, no significant association between SBP and HRV was reported; however, it is important to note that only SBP was assessed (Henje Blom et al., 2009). The present study assessed both SBP and DBP and found DBP was more influential than SBP at predicting VLF, SDNN, SDNNi, and rMSSD. Ultimately, heart rate remained the most significant covariate for all HRV parameters and thus, should be considered in future research. To the best of our knowledge, no studies have previously evaluated SBP and DBP as covariates of HRV among healthy school-aged children.

Age, Sex, and Puberty. Prior research demonstrates that heart rate decreases across childhood and this decline is thought to begin between the ages of 5 to 10 years (Finley et al., 1987, 1995), increases slightly between ages 15 to 18 years (Lenard et al., 2004), and then significantly declines into young adulthood (Umetani et al., 1998).

Given that many pediatric studies do not control for heart rate, age-related changes in HRV may very well be attributable to age-related changes in heart rate (Fleming et al., 2011; Walsh et al., 2005). In fact, when age was simultaneously entered with other covariates (post-hoc analyses), heart rate remained significant and age became non-significant. In the present study, age was a significant covariate for parasympathetic-driven variables (e.g., rMSSD, pNN50). These results are comparable to past findings, which reported greater rMSSD and pNN50 values among 7 to 10 year old children compared to 11 to 14 year old youth (Lenard et al., 2004).

Consistent with previous studies (Faulkner et al., 2003; Henje Blom et al., 2009; Reed, Warburton, Whitney, & McKay, 2006; Silvetti et al., 2001; Wang et al., 2005), sex differences emerged. Boys manifested lower heart rate and greater HRV, particular those reflecting parasympathetic activity. Girls evidenced higher heart rate and greater sympathovagal imbalance. This may be partly attributable to pubertal onset, given that girls typically enter puberty 2 years earlier than boys (Rogol et al., 2000).

Relatedly, more advanced pubertal development, in both gonardarche and adrenarche stages, was associated with increased sympathovagal imbalance and reduced parasympathetic activity across time- and frequency-domain HRV variables. These results are similar to previous research and support the notion that the timing of pubertal development coincides with the emergence and maturation of neural autonomic mechanisms that reach their peak level during adolescence (Chen et al., 2012; Dorn, Dahl, Woodward, & Biro, 2006; Faulkner et al., 2003; Lenard et al., 2004; Ordaz & Luna, 2012). It has been posited that gonadal hormones (e.g., estrogen and testosterone) may interact with serotonin transporter genotype to yield sex-specific increases in blood

pressure (in males) and heart rate (in females) through sympathetic activation with similar results documented in animals (McCabe, Porges, & Carter, 1981; McEwen & Gianaros, 2010; Spear, 2000).

Sleep. Notably, bed-times on school and weekend nights, average sleep duration, and wake after sleep onset were significant covariates in HRV. Consistent with the limited pediatric data, later bed-times and shorter sleep duration were associated with reduced HRV and wake after sleep onset was associated with increased sympathovagal imbalance (Massin et al., 2000; Rodriguez-Colon et al., 2011). These results are inconsistent with a previous study that found no association between HRV and later sleeping patterns (Henje-Blom et al., 2009). These discrepancies are most attributable to the sample they used (i.e., adolescents), and the method used to assess sleep patterns (i.e., a single question on the frequency of "sleeping past midnight"), which may not have fully captured the complexity and multidimensionality of sleep construct (Jarrin, McGrath, & Drake, submitted).

Prominent developmental changes in sleep patterns have been documented among children aged 10 to 13 years, as they go to bed later, have shorter sleep duration, and have increased sleep problems (e.g., difficulty initiating and maintaining sleep; Carskadon et al., 1997; Carskadon, Vieira, & Acebo, 1993; Laberge et al., 2001). These age-related changes in timing of sleep may be influenced by contextual (i.e., school start times) and physiological factors (i.e., changes in circadian system) that are particularly pertinent during this developmental period (Dahl & Carskadon, 1995; Myers & Badia 1995). Interestingly, advanced pubertal development is associated with major changes in sleep patterns, particularly among females (e.g., later bed-times, sleep problems; Carskadon et

al., 1993; Laberge et al., 2001), which also overlap with the present findings. Taken together, female sex, advanced pubertal development, and later bed-times and shorter sleep duration were associated with reduced HRV.

Anthropometrics. While past pediatric studies report a significant association between reduced HRV and greater obesity measures (c.f., Kaufman et al., 2007; Martini et al., 2001; Rodriguez-Colon, Bixler, Li, Vgontzas, & Liao, 2011), the present study did not observe similar trends. Contrary to expectations, larger waist circumference was associated with reduced VLF. The physiological mechanism underlying VLF is disputed; however, it is proposed to reflect the renin-angiotensin-aldosterone system (Taylor, Carr, Myers, & Eckberg, 1998), which when activated, has been implicated in the promotion of increased fat mass (Haynes, Morgan, Walsh, Mark, & Sivitz, 1997). Although, several pathophysiological mechanisms are implicated in the renin-angiotensin-aldosterone system (i.e., pro-sclerotic and pro-fibrotic cytokines, transforming growth factor-b, promotion of endothelial dysfunction; Brewster, Setaro, & Perazella, 2003; Steckelings, Rompe, Kaschina, & Unger, 2009), and thus, the putative underlying mechanisms between VLF and obesity are not well understood. Alternatively, the disparate findings may be attributed to the duration of obesity, which has been previously suggested to influence autonomic cardiovascular control (Rabbia et al., 2003). Further, many studies that investigate HRV and obesity among youth, do not commonly report on VLF (c.f., Kaufman et al., 2007; Martini et al., 2001; Riva et al., 2001; Rodriguez-Colon et al., 2011).

Physical Activity. Similar to past studies, there was a significant association between physical activity and diminished sympathovagal imbalance in children (c.f.,

Chen et al., 2012; Henje Blom et al., 2009; Nagai & Moritani, 2004) . Exercise is thought to promote electrical stability, stimulating coordinated contractions in the ventricles of the heart (Berntson et al., 1997; Stein & Kleiger, 1999). Conversely, sedentary behavior is associated with increased sympathovagal imbalance, which confers risk for ventricular fibrillation, a condition that causes disruptions in the contractions of the ventricles of the heart and prevents blood circulation (Billman & Hoskins, 1988; Hull et al., 1990; Molgaard, Sorensen, & Bjerregaard, 1991). As such, it may be advantageous to consider physical activity as a potential covariate in future studies investigating HRV.

Additional Covariates. Lastly, earlier recording time and longer processing time were associated with greater HRV parameters and sympathovagal imbalance, except rMSSD and pNN50. These findings may reflect a circadian pattern in HRV, which inherently also reflects circadian variations in the autonomic nervous system (Yamasaki, Kodama, & Matsuhisa, 1996). It is posited that HRV parameters decrease significantly throughout the day and increase during the night, while sympathovagal imbalance shows the reverse pattern (Guo & Stein, 2002). Massin and colleagues (2000) also observed circadian variations within a pediatric sample.

Further, longer processing time was found to be associated with greater HRV parameters. Longer processing times may be related to the editing procedures that remove artifacts that can influence HRV values dramatically (Berntson et al., 1997). Not surprisingly, recording and signal processing issues have been identified as important factors associated with the discrepancies found in a systematic review of short-term HRV values in adults (Nunan et al., 2010). As such, these results further highlight the significance of standardized recording and editing protocols when analyzing HRV

parameters (Bernsten et al., 1997; Jarrin et al., 2012; Nunan et al., 2010; Task Force, 1996).

In summary, a substantive proportion of the variance in HRV can be accounted for by sex, heart rate, and blood pressure, a finding consistent with the adult literature. Importantly, other developmentally relevant covariates during childhood that influence HRV parameters include pubertal status, bed-time, sleep duration, sleep-related problems, sampling time, and total duration of recording. Likewise, when multiple covariates were assessed collectively, heart rate, gonardarche pubertal status, DBP, start times, and total processing times were most influential. Given that each HRV parameter had different covariates emerge as relevant, it is strongly recommended to carefully consider which covariates are most appropriate when investigating a particular HRV parameter in children.

Strengths, Limitations, and Future Recommendations

We acknowledge limitations in the present study. First, the sample was limited to 9 to 11 year old children, limiting the generalizability of the results. However, unlike previous cross-sectional studies, with small sizes, wide age categories, or restrictive clinical sample, this was the first study to present normative HRV values in a large, population-based sample of healthy children. Notably, HRV values were largely similar to past studies assessing school-aged children (Table 1). Longitudinal designs with repeated measures of HRV and covariates are necessary to more comprehensively understand the complex nature of the ANS across the childhood and adolescence developmental spans of the lifecourse.

Second, only time-and frequency-domain HRV variables were evaluated. Other methods to analyze HRV exist (e.g., geometric, nonlinear), which provide supplementary information on the dynamics and complexity of the regulation of the ANS (Task Force, 1996; Voss, Schulz, Schroeder, Baumert, & Caminal, 2009). However, time- and frequency-domain variables are the most commonly used HRV parameters in pediatric studies and were analyzed in adherence to the Task Force (1996) methodological and technical recommendations. Thus, to further facilitate comparison and the synthesis of findings across studies, the present analyses were limited to time- and frequency-domain variables.

Third, only short-term recordings were evaluated. Compared to long-term recordings, short-term recordings are considered a less sensitive technique in capturing frequency-domain variables, such as VLF and ultra low-frequency (ULF; Task Force, 1996). Long-term recording, however, are capable of monitoring and capturing continuous fluctuations in HRV under normal day-to-day routines, which provide more ecological validity compared to short-term recordings. However, because short-term recordings are based on brief acquisition time of ECG data (e.g., 1 min) and can capture *most* time- and frequency-domain variables, they do provide practical advantages over long-term recordings (Task Force, 1996). Short-term recordings are acquired under standard physiological states (e.g., comfortably sitting in a relaxed position, limiting body movements), during standardized protocols, and may yield less biased recordings, that are free of missing data and artifacts (Task Force, 1996). The majority of pediatric studies only report short-term recordings (refer to Table 1). Nonetheless, future studies should accrue population-based normative values for short- and long-term recordings (i.e., 24-

hours; e.g., Poincare plots, etc.) for the commonly used time- and frequency-domain variables.

Fourth, many of the covariates were based on subjective parental- or self-report (e.g., pubertal status, sleep duration). Other possible covariates were not objectively assessed (e.g., physical activity, sleep fragmentation, body fat composition; Liao et al., 2010; Wang et al., 2005). Yet, these subjective measures have demonstrated reliability and validity with objective measures in past studies (e.g., Netherton et al., 2004; Wolfson et al., 2003). Further, most physiological covariates (e.g., heart rate, blood pressure) were objectively assessed using advanced equipment, by trained staff, following standardized protocols. Nevertheless, future studies should consider testing objectively assessed measures as well as other covariates that may be particularly relevant for children (e.g., sleep-wake patterns, screen time).

Conclusion

Normative time- and frequency-domain HRV values for a large, population-based sample of 10-year-old children were provided in the present paper. Of the developmentally relevant covariates tested, sex, heart rate, blood pressure, pubertal status, sleep, physical activity, as well as time and length of ECG recording significantly accounted for variance in the HRV parameters and should be measured and controlled for (methodologically or statistically) when analyzing HRV in children. Researchers should be prudent in identifying appropriate covariates, depending on the HRV parameter of interest, given their research question. Altogether, these data have potential implications to advance the field, as these referent values may facilitate comparison and synthesis of previously reported HRV data among pediatric studies.

Acknowledgements: We wish to thank the children and their families for their longstanding commitment to this research project. This study was funded by the Canadian Institute of Health Research (CIHR) Grants Numbers 00309MOP-123079 and HDF-70335. Data were collected by the Institut de la Statistique du Québec, Direction Santé Québec and the Research Unit on Childhood Psychosocial Maladjustment, Université de Montréal. The IRSPUM, CRCHUM, and Centre de Recherche du CHU Ste-Justine received infrastructure funding from the Fonds de la Recherche en Santé du Québec. These funding agencies were not involved in the study design, data analyses, data interpretation or manuscript writing and submission processes. J.J. McGrath holds a CIHR New Investigator Award, D.C. Jarrin holds a Fonds de la Recherche en Santé du Québec Doctoral Fellowship (FRSQ) and Health Professional Student Research Award, P. Poirier is a senior clinician-scientist of the FRSQ, G. Paradis holds a CIHR Applied Public Health Research Chair.

Table 1

Means and Standard Deviations of Short-term Heart Rate Variability Values across Studies Using Healthy Children

Author, Year	Recording Time (min)	N	Mean Age	Mean HR (beats/min)	Mean RR (ms)	Time-Domain Variables: SDNN (ms)	rMSSD (ms)	pNN50 (%)	Frequency-Domain Variables: LF	HF	RATIO	VLF
3 to 5 minute recordings												
McCarty 1999	4	30 ♂=36.6%	12-13 12.2	86.80 (10.24)	705.87 (86.32)	58.57 (21.38)	---	---	1215.75(799.09)$_{ms^2}$ 6.91(64)$_{ln}$	1029.78(915.21)$_{ms^2}$ 6.66(74)$_{ln}$	---	997.24 (1013.71)$_{ms^2}$ 6.46(1.00)$_{ln}$
Yang 2001	4	30 ♂=70%	4-10 6 (1.3)	---	---	---	---	---	43.94 (16.39)$_{nu}$	30.5 (10.93)$_{nu}$	1.87 (1.67)	---
Henje Blom 2009	4	71 ♂=33%	16.5	74.5 (9.63)	---	4.11 (0.35) $_{ln}$	---	---	5.88 (0.93) $_{ln}$	6.03 (0.90) $_{ln}$	---	---
†Nagai 2003	4.5	42 ♂=42.8%	6-12 9 (0.3)	84.3 (1.0)	---	---	---	---	6.42 (0.05)$_{lnms^2}$	6.34 (0.07)$_{lnms^2}$	---	---
¥Winsley 2003	5	12 ♂=41.6%	11-12 12.8 (0.30)	---	---	64 (27)	56 (28)	28 (19)	758(632)$_{ms^2}$ 37(12)$_{nu}$	920(802)$_{ms^2}$ 53(14)$_{nu}$	0.81 (0.50)	---
†Nagai 2004	5	24 ♂=33%	9.4 (1.8)	---	---	---	---	---	---	6.50(0.40)$_{lnms^2}$	---	---
Wang 2005	5	234 ♂=47.8%	16 (2)	---	♂=933 (150) ♀=853(115)	♂=72(34) ♀=61(25)	♂=72(43) ♀=64(37)	---	♂=296(289)$_{lnHz}$ ♀=244(227)$_{lnHz}$	♂=421(541)$_{lnHz}$ ♀=374(374)$_{lnHz}$	♂=1(0.8) ♀=1(0.9)	---
Reed, 2006	5	62 ♂=48.3%	9-11 ♂=10.2(0.6) ♀=10.5(0.6)	♂=77.9 (12.1) ♀=80 (10.1)	---	♂=60.17 (30.01) ♀=60.81 (35.91)	♂=65.18 (38.21) ♀=64.12 (53.17)	---	♂=3.57(0.41)$_{ln}$ ♀=3.79(0.39)$_{ln}$ ♂=38.71(14.81)$_{ms^2}$ ♀=47.62(17.41) $_{ms^2}$	♂=4.07(0.28)$_{ln}$ ♀=3.89(0.36)$_{ln}$ ♂=61.25(14.81)$_{ms^2}$ ♀=52.33(17.39)$_{ms^2}$	♂=4.11 (0.68) ♀=4.51 (0.74)	---
¥Chen 2008	5	107 ♂=46.7%	8-12 10.4 (1.6)	---	---	---	---	---	6.3 (0.6)$_{lnms^2}$	5.2 (0.9)$_{lnms^2}$	1.2 (0.7)$_{ln}$	---
†Longin 2009	5	100 ♂=49%	6-15 10.32 (2.58)	---	---	66.7 (36.3)	---	---	74.1 (27.3)$_{ms^2}$	217.6 (117.4)$_{ms^2}$	---	---
Gamelin 2009	5	16 ♂=43.7%	9.3 (1.2)	80.8 (6.8)	756.3 (68.1)	70.2 (33.9)	67.4 (32.8)	35.3 (19.2)	5.7(1.1)$_{ln}$ 42(12.4)$_{nu}$	6.1(0.8)$_{ln}$ 58(12.4)$_{nu}$	-0.4 (0.6)$_{ln}$	---

Author, Year	Recording Time(min)	N	Mean Age	Mean HR (beats/min)	Mean RR (ms)	Time-Domain Variables: SDNN (ms)	rMSSD (ms)	pNN50 (%)	Frequency-Domain Variables: LF	HF	RATIO	VLF
Dietrieta 2010	5	57 ♂=52.6%	10-13 11.2 (0.7)	73.06 (10.07)	---	4.22 (0.52)	---	---	6.87 $(0.99)_{ln}$	7.58 $(1.31)_{ln}$	---	---
Latchman 2011	5	19	7-12 9.2 (1.4)	78 (13.1)	---	---	---	---	$7.2(0.5)_{RRln}$	$8.2(1.1)_{RRln}$	0.54 (0.3)	---
Chen 2011	5	87 ♂=50.6%	9-13 10.6 (1.5)	---	---	---	---	---	$5.9(0.9)_{lnms}{}^2$	$5.6(1.3)_{lnms}{}^2$	---	---
Sharma 2012	5	30 ♂=50%	8-18 11.66 (2.5)	---	---	63.67 (29.91)	67.17 (42.22)	1634.84 (1066.03)	$1453(1856.79)_{ms}{}^2$ $37.41(18.13)_{nu}$	$2389.44(2919.42)_{ms}{}^2$ $47.67(19.46)_{nu}$	1.12 (1.1)	---
¥Vykunta Raju 2012	5	40 ♂=45%	2-15 9.1 (3.3)	---	---	---	---	13 0.17-76	$602_{ms}{}^2$ 91-2880	$1303_{ms}{}^2$ 91-10707	0.56 0.13-9.65	---
Moodithaya 2012	5	60 ♂=50%	6-11 9.4 (0.32)	---	---	---	---	---	$960\ (92)_{ms}{}^2$	$1603\ (163)_{ms}{}^2$		
9 to 60 minute recordings												
†Wawryk 1997	9	108 ♂=58.3%	8-17 12.8 (3.2)	79.2 (11.8)	---	---	---	---	2.99 $(0.38)^{log}{}_{10}$	3.18 $(0.51)^{log}{}_{10}$	-0.19 $(0.28)^{log}{}_{10}$	---
Yeragani 2001	10	15	10.5 (2.3)	---	---	---	---	---	$2.3(0.97)_{pwr}$ $1.08(1.08)^{abs}{}_{power}$	$2.4(0.85)_{power}$ $1.58(1.08)^{abs}{}_{power}$	0.69 (0.39)	$2.5(1.1)_{pwr}$ $1.97(0.95)^{abs}{}_{pwr}$
†Lenard 2004	10	34 ♂=44.1%	7-10 8.2 (0.2)	---	---	64 (4)	64 (6)	28 (3)	$42.8(2.9)_{nu\%}$ $1011(122)_{ms}{}^2$	$56.1(3.4)_{nu\%}$ $1559(332)_{ms}{}^2$	---	---
†Lenard 2004	10	36 ♂=55.5%	11-14 12.1 (0.2)	---	---	61 (4)	55 (5)	26 (4)	$44.1(3.2)_{nu\%}$ $928(118)_{ms}{}^2$	$53.5(3.3)_{nu\%}$ $1410(254)_{ms}{}^2$	---	---
Kaufman 2007	15	36 ♂=52.7%	10-13 11.5 (0.10)	---	0.847 (0.02)s	0.092 (0.01)s	110.8 (3.9)	---	$51.1(2.1)_{nu\%}$ $3.9(0.05)_{lnnu\%}$	$48.9(2.1)_{nu\%}$ $3.85(0.05)_{lnnu\%}$	1.21 (0.12)	---
Vanderlai 2010	20	61 ♂=50.8%	8-12 10.49 (1.39)	---	---	---	---	---	$384.40(211.7)_{ms}{}^2$ $60.80(11.18)_{nu}$	$251.60(155.0)_{ms}{}^2$ $39.20(11.18)_{nu}$	1.74 (0.74)	---
Acharya 2004	20	25	5-15	---	---	92.96 (48.60)	88.48 (65.65)	12.91 (8.46)	---	---	1.42 (1.05)	---
Kwok 2011	60	51	2-16 9.59 (2.96)	---	---	1013.5 (192.5)	98.2 $(38.1)_{ln}$	51.1 (29.2)	$2859(1998)_{ms}{}^2$ $0.25(0.05)_{nu}$	$2326(1766)_{ms}{}^2$ $0.20(0.08)_{nu}$	1.49 (0.82)	$4698(2838)_{ms}{}^2$ $0.42(0.08)_{nu}$

Note. ¥ =No information on frequency bandwidths. † = Frequency bandwidths differ from Task Force (1996) recommendations.

Table 2

Means and Standard Deviations of Long-term Heart Rate Variability Values (24 hrs) across Studies Using Healthy Children

					Time-Domain Variables					Frequency-Domain Variables			
Author, Year	N	Mean Age	Mean HR (beats/min	Mean RR ms)	SDNN (ms)	SDANN (ms)	SDNNi (ms)	rMSSD (ms)	pNN50 (%)	LF	HF	RATIO	VLF
†Finley 1995	10	10-12	84 (13)	18 (1.5)	---	---	---	---	---	11.1 (2.8)$_{bts/min}^2$	11.6 (3.4)$_{bts/min}^2$	1.22(0.25)	---
Yeragani 1997	10 ♂=50%	11.1 (12.1)	92.9 (11.2)	---	---	---	---	---	---	7.2(2.8)$_{\%tp}$ 2(0.4)$_{lnbpm}^2$	3.3(1.2)$_{\%tp}$ 1.3(0.5)$_{lnbpm}^2$	2.3 (0.8)	---
Umentani 1998	30 ♂=53.3%	10-19	80 (10)	---	176 (38)	159 (35)	81 (20)	53 (17)	25 (13)	---	---	---	---
Pikkujämsä 1999	27	1-14 8 (5)	---	678 (105)	140 (46)	---	---	---	---	6.85 (0.97)$_{ln}$	6.83 (1.12)	---	9.39 (0.81)$_{ln}$
Heragu 1999	11	6-11	---	657(42)	117 (16)	98 (17)	59 (8)	---	---	14 (15) $_{beats/min}$	17 (12)$_{beats/min}$	0.8 (0.6)	---
Batten 2000	36 ♂=100%	10-16 12	---	---	185 (0.14)	160 (0.83)	90 (0.69)	64 (0.02)	31 (0.03)	1926 (55)$_{ms}^2$	1644 (33)$_{ms}^2$		3568 (23)$_{ms}^2$
Kazuma 2000	Spring: 28 Summer: 16 Autumn: 31 Winter: 20	5-15 9.5 (3.5)	---	---	---	---	---	---	---	1380(796)$_{ms}^2$ 1349(478) $_{ms}^2$ 1428(894) $_{ms}^2$ 988(543)$_{ms}^2$	1386(945)$_{ms}^2$ 1493(705)$_{ms}^2$ 1336(830)$_{ms}^2$ 995(577)$_{ms}^2$	1.51 (0.52) 1.65 (0.63) 1.66 (0.63) 1.71 (0.81)	
Han 2000	39	6-18	95.3 (15.3)	637.1 (95.3)	148.3 (38.9)	129 (35.5)	78.7 (21.5)	54.1 (13.3)	26.5 (9.2)	7.04 (0.57)$_{lnms}^2$	6.54 (0.57)$_{lnms}^2$	1.08 (0.04)	---
Han 2000	11	9-11 9.1 (1.2)	96.3 (8.8)	627.6 (58.9)	139.9 (21.5)	118.3 (20.3)	79.7 (17.5)	53.1 (9.9)	26.5 (6.8)	7.07 (0.44)$_{lnms}^2$	6.63 (0.48)$_{lnms}^2$	1.07 (0.06)	---
Dundaroz 2001	22 ♂=63.6%	7-14 9.1 (1.2)	---	---	142 (29)	122 (22)	---	89 (45)	34 (13)	---	---	---	---
Riva 2001	14	12.9 (1.6)	---	---	153.9 (47.3)	127.7 (49.1)	---	56.7 (14.1)	28.7 (8)	23.7 (3.4)	16.3 (4.5)	1.5 (0.4)	---
†Fujiwara 2001	26 ♂=53.8%	10 (3)	---	---	---	---	---	---	---	19.8 (3.8)%	15.2 (5.8)%	1.46 (0.60)	---
Martini 2001	13 ♂=53.8%	13.1 (1.7)	74.4 (6)	786.7 (63.5)	149 (51)	123 (53)	---	56.5 (15.8)	28 (9)	25.3 (2.5)$_{nu}$	17.7 (5.3)$_{nu}$	1.5 (0.4)	---
Silvetti 2001	28 ♂=64.2%	6-10 ♂=7.0 (1.4) ♀=7.4 (1.1)	---	---	♂=154(33) ♀=133(32)	♂=119(30) ♀=109(19)	♂=93(33) ♀=75(33)	♂=97(65) ♀=75(50)	♂=31(17) ♀=24(11)	---	---	---	---

					Time-Domain Variables					Frequency-Domain Variables			
Author, Year	N	Mean Age	Mean HR (beats/min	Mean RR (ms)	SDNN (ms)	SDANN (ms)	SDNNi (ms)	rMSSD (ms)	pNN50 (%)	LF	HF	RATIO	VLF
Silvetti 2001	37 ♂=64.2%	11-15 ♂=13.1 (1.6) ♀=13.6 (1.4)	---	---	♂=183 (40) ♀=155 (45)	♂=155(39) ♀=123(41)	♂=91(19) ♀=88(25)	♂=71(19) ♀=77(30)	♂=28(10) ♀=28(12)	---	---	---	---
Kazuma 2002	18	6-7 6.3(0.5)	---	661 (90)	---	---	---	---	---	820 (438)$_{ms}^2$	863 (880)$_{ms}^2$	1.37 (0.64)	---
Kazuma 2002	38	10-12 10.9 (0.9)	---	756 (79)	---	---	---	---	---	1360 (624)$_{ms}^2$	737 (465)$_{ms}^2$	2.34 (1.10)	---
Kazuma 2002	14	8-9 8.7(0.5)	---	707 (73)	---	---	---	---	---	1062 (397)$_{ms}^2$	777 (514)$_{ms}^2$	1.71 (0.86)	---
Kazuma 2002	53	6-12 9.2 (2.1)	---	734 (91)	---	---	---	---	---	1196(584)$_{ms}^2$	808(547)$_{ms}^2$	1.84 (0.90)	---
Faulkner 2003	70 ♂=37.1%	13-18 15(1.6)	---	---	♂=177.2 (36.5) ♀=149.3 (35.9)	♂=144.6 (34.4) ♀=121.8 (33.8)	---	♂=61.8 (16.7) ♀=49.2 (15.7)	♂=29.3 (10.1) ♀=22 (10.4)	♂=7.39(0.37)$_{ln}$ ♀=7.01(0.60)$_{ln}$	♂=6.65 (0.61)$_{ln}$ ♀=6.29 (0.70)$_{ln}$	---	---
Faulkner 2003	43 ♂=43.1%	13-14 13.83 (0.57)	---	---	♂=178.16 (39.59) ♀=145.79 (34.83)	♂=149.52 (38.59) ♀=120.33 (34.90)	♂=92.68 (18.76) ♀=76.29 (21.11)	♂=60.68 (15.35) ♀=50.08 (18.29)	♂=28.83 (9.79) ♀=22.44 (12.37)	♂=7.40(0.39)$_{ln}$ ♀=7.05(0.64)$_{ln}$	♂=6.67 (0.59)$_{ln}$ ♀=6.34(0.77)$_{ln}$	---	---
Unalacak 2004	20 ♂=60%	6-13 8 (1.9)	---	---	120 (28)	106 (37)	---	33 (9)	---	266 (110)$_{ms}^2$	160 (109)$_{ms}^2$	1.44 (0.36)	627 (387)$_{ms}^2$
Babaoglu 2011	54 ♂=31.4%	6-18 11.2 (3.4)	81.9 (14.2)	689 (80)	137 (33)	122 (32)	62 (20)	44 (10)	24(9)	6.3 (0.5)$_{ln}$	6.7 (0.6)$_{ln}$	1.06 (0.06)	---
¥Karacan 2011	37 ♂=59%	5-14 10.7	86.3 (9.1)	693.2 (72.2)	143.8 (38.5)	121 (35.1)	75.7 (19.6)	54.5 (19.5)	24.1 (10.6)	1137.4 (557)$_{ms}^2$ 54.9 (12.5)$_{nums}^2$	823.8(438.5)$_{ms}^2$ 38.2(6.7)$_{nums}^2$	1.61 (0.51)	3504.3 (1951.2)$_{ms}^2$
Buchhorn 2012	19 ♂=52.6%	10.8 (3.5)	84.7 (1.8)	---	---	---	---	44.5 (10.1)	21.5 (9.0)	---	---	---	---

Note. ¥ =No information on frequency bandwidths. ┼ = Frequency bandwidths differ from Task Force (1996) recommendations.

Table 3

Mean and Standard Deviation on Heart Rate Variability Time-and Frequency-Domain Variables in Children

	Boys		Girls		Total	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Age (years)	10.22	0.29	10.20	0.29	10.21	0.29
Mean RR (ms)	691.01	71.71**	669.50	63.32	679.49	68.16
Mean HR (beats/min)	79.08	10.50**	81.12	9.79	80.17	10.17
Time-Domain						
SDNN (ms)	89.38	26.12**	84.35	23.08	86.69	24.66
SDANN (ms)	42.27	17.14**	39.33	15.19	40.70	16.18
SDNNi (ms)	77.38	22.92**	72.96	20.98	75.01	22.00
rMSSD (ms)	45.05	13.66***	41.05	12.88	42.91	13.39
pNN50 (%)	21.31	11.37***	18.26	10.79	19.68	11.16
Frequency-Domain						
VLF $_{(ms^2)}$	1621.86	1139.46**	1448.30	840.83	1528.88	994.02
ln VLF	7.21	0.59*	7.13	0.53	7.17	0.56
LF $_{(ms^2)}$	1587.68	1040.06**	1411.16	934.35	1493.11	988.29
ln LF	7.18	0.61**	7.07	0.59	7.12	0.60
HF $_{(ms^2)}$	896.29	684.40**	770.65	622.22	828.99	654.52
ln HF	6.53	0.75***	6.37	0.76	6.44	0.76
LF:HF ratio	2.05	0.81**	2.18	0.92	2.12	0.87

Note. SDNN = standard deviation of all normal sinus RR intervals; SDANN = standard deviation of the averaged normal sinus RR intervals for all 5-min segments; SDNNi = mean of the standard deviations of all normal sinus RR intervals for all 5-min segments; rMSSD = root-mean-square of the successive normal sinus RR interval difference; pNN50 = percentage of successive normal sinus RR intervals 50 ms; VLF = Very low frequency (0.0033-0.04 Hz); ln=log-transformed value; LF = Low frequency (0.04-0.15 Hz); HF = High frequency (0.1500-0.4 Hz); ms = milliseconds. Sex difference at

* $p < .05$; ** $p < .01$; *** $p < .001$

Table 4

Normative Heart Rate Variability Percentile Values While Controlling for Age and Heart Rate for Boys and Girls

	Boys					Girls				
	5th	25th	50th	85th	95th	5th	25th	50th	85th	95th
Time-Domain										
SDNN (ms)	71.14	83.92	95.46	111.66	124.87	76.36	89.87	100.27	116.99	128.46
SDANN (ms)	15.06	20.01	24.53	31.07	36.32	17.80	23.27	27.22	33.91	38.80
SDNNi (ms)	83.99	95.77	106.68	121.63	134.36	89.00	101.30	110.77	126.27	136.48
rMSSD (ms)	41.22	49.00	55.99	65.88	73.86	45.42	53.63	59.94	70.11	77.08
pNN50 (%)	31.73	38.01	43.74	51.76	58.32	34.85	41.58	46.69	55.00	60.72
Frequency-Domain										
VLF (ms^2)	2936.76	3363.01	3756.21	4292.96	4749.61	3131.39	3573.67	3914.11	4474.70	4843.68
ln	2.63	2.92	3.18	3.54	3.87	2.72	3.03	3.26	3.62	3.85
LF (ms^2)	3882.05	4316.63	4713.27	5270.73	5765.80	4079.88	4541.83	4892.35	5448.84	5801.63
ln	3.35	3.65	3.93	4.32	4.66	3.47	3.80	4.03	4.43	4.65
HF (ms^2)	1668.35	1946.63	2202.25	2559.92	2857.18	1797.22	2094.63	2322.35	2695.92	2945.90
ln	2.93	3.31	3.66	4.15	4.56	3.11	3.51	3.82	4.31	4.64
LF:HF ratio	0.01	0.07	0.16	0.35	0.50	0.02	0.09	0.19	0.42	0.60

Note. SDNN = standard deviation of all normal sinus RR intervals; SDANN = standard deviation of the averaged normal sinus RR intervals for all 5-min segments; SDNNi = mean of the standard deviations of all normal sinus RR intervals for all 5-min segments; rMSSD = root-mean-square of the successive normal sinus RR interval difference; pNN50 = percentage of successive normal sinus RR intervals 50 ms; VLF = Very low frequency (0.0033-0.04 Hz); ln = log-transformed value; LF = Low frequency (0.04-0.15 Hz); HF = High frequency (0.1500-0.4 Hz); ms = milliseconds.

Table 5

Covariates on Heart Rate Variability Frequency- and Time- Domain Variables in Children

Covariates	Descriptives	Standardized Beta Coefficients								
		Time-Domain Variables					Frequency-Domain Variables			
	Mean; SD (n;%)	*SDNN*	*SDANN*	*SDNNi*	*rMSSD*	*pNN50*	*VLF (ln)*	*LF (ln)*	*HF (ln)*	*Ratio*
Age (years)	10.21; 0.29	0.05	0.05	0.03	**0.06***	**0.06***	0.00	-0.02	0.01	**-0.06***
Sex (Male)	(481; 46.4%)	**0.10****	**0.09****	**0.10****	**0.14*****	**0.13*****	**0.06***	**0.08****	**0.10*****	**-0.08****
SBP (mmHg)	97.00; 10.06	**-0.17*****	**-0.15*****	**-0.17*****	**-0.15*****	**-0.13*****	**-0.18*****	**-0.18*****	**-0.14*****	0.04
DBP (mmHg)	61.65; 8.82	**-0.18*****	**-0.14*****	**-0.18*****	**-0.16*****	**-0.15*****	**-0.15*****	**-0.16*****	**-0.13*****	0.02
Heart Rate (beats/min)	80.17; 10.17	**-0.66*****	**-0.40*****	**-0.69*****	**-0.74*****	**-0.72*****	**-0.65*****	**-0.64*****	**-0.64*****	**0.28*****
Start Time (hr:min)	10:41; 3:13	**-0.19*****	**-0.20*****	**-0.17*****	-0.04	-0.01	**-0.27*****	**-0.18*****	**-0.06***	**-0.14*****
Processing time (min)	69.24; 21.67	**0.07***	**0.19*****	0.02	-0.05	**-0.07***	**0.14*****	**0.06***	-0.02	**0.12*****
Pubertal Development										
Gonardarche	1.85; 0.64	-0.03	0.00	-0.04	**-0.10****	**-0.10****	-0.02	-0.02	**-0.07***	**0.12****
Adrenarche	1.73; 0.69	-0.01	0.02	-0.02	**-0.08****	**-0.08****	-0.01	-0.01	**-0.06***	**0.11****
Anthropometric Measures										
BMI Z-score (percentile)	59.93; 27.79	0.00	-0.04	0.02	0.05	0.05	0.00	0.03	0.03	-0.00
Waist Circumference(cm)	64.34; 9.40	-0.04	**-0.05**$^{+}$	-0.02	0.00	0.00	**-0.07***	-0.04	-0.01	-0.02
Sleep Measures										
School Bed-time	20:31; 0:31	**-0.07***	**-0.08***	**-0.06**$^{+}$	-0.02	-0.01	**-0.09****	**-0.08***	-0.05	-0.02
Weekend Bed-time	21:36; 0:41	**-0.08***	**-0.09***	**-0.07***	-0.02	-0.02	**-0.09****	**-0.07***	**-0.06**$^{+}$	0.01
Average Sleep Duration	9:52; 0:40	**0.07***	**0.06**$^{+}$	**0.06**$^{+}$	0.05	0.05	**0.08***	**0.06**$^{+}$	**0.06**$^{+}$	-0.02
WASO (Yes)	(91; 11%)	-0.00	0.03	-0.02	-0.04	-0.04	-0.01	-0.02	**-0.06**$^{+}$	**0.10****
Physical Activity (Yes)	(32; 3.6%)	-0.01	**-0.06**$^{+}$	-0.05	0.02	0.03	-0.06	-0.02	0.03	**-0.08****

Note. SBP = systolic blood pressure; DBP = diastolic blood pressure; SDNN = standard deviation of all normal sinus RR intervals; SDANN = standard deviation of the averaged normal sinus RR intervals for all 5-min segments; SDNNi = mean of the standard deviations of all normal sinus RR intervals for all 5-min segments; rMSSD = root-mean-square of the successive normal sinus RR interval difference; pNN50 = percentage of successive normal sinus RR intervals 50 ms.; VLF = Very low frequency (0.0033-0.04 Hz); Ln = log-transformed value; LF = Low frequency (0.04-0.15 Hz); HF = High frequency (0.1500-0.4 Hz); ms = milliseconds; WASO = wake after sleep onset. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; $+p < .08$

TRANSITION TO MANUSCRIPT 3

The objectives of manuscript 2 were to establish normative HRV values of traditional time-and frequency-domain variables in children and to assess potential developmentally-relevant covariates. Manuscript 2 is an original contribution to the field that provides normative HRV values for a large, population-based cohort of 10-year-old children. It is anticipated that these referent values will foster comparisons of HRV values across pediatric studies.

In addition, several covariates were identified for child HRV parameters. Manuscript 2 evaluated *multiple* developmentally-relevant covariates of HRV, both singularly and collectively. Results revealed that heart rate, gonardarche pubertal status, and DBP are pertinent when analyzing HRV in children. It is suggested that these be included as standard covariates when using HRV in children. Taken together, the results of manuscript 1 and 2 established a methodological foundation for comparing and evaluating HRV in children, a prerequisite to my overarching aim of examining sympathovagal balance in the relation between sleep and child obesity.

In adults, there is convincing evidence that sleep duration is significantly related to HRV, as demonstrated by experimental studies (Speigel et al., 1999, 2004; Van Cauter, 2007). One particularly intriguing finding is that HRV seems to be associated with other sleep dimensions beyond sleep duration, such as sleep patterns and disturbances. While these sleep dimensions partly overlap, they are associated with unique physiological mechanisms. Thus, it was necessary to determine their independent relation with obesity as this may have informed a more comprehensive understanding of putative pathophysiological mechanisms linking sleep and obesity. Interestingly, most pediatric

studies examining the link between sleep and obesity almost exclusively focus on sleep duration as the sole indicator of sleep. This insular approach may be misguided given that sleep is a dynamic multidimensional construct that extends beyond sleep duration.

Manuscript 3 investigated whether other sleep dimensions, beyond sleep duration, were associated with childhood obesity. Specifically, the aim of manuscript 3 was to test the associations between sleep patterns (e.g., bed- and rise-times) and sleep disturbances (e.g., parasomnias) with multiple indicators of obesity, while controlling for sleep duration and obesity-related covariates in a sample of healthy youth.

MANUSCRIPT 3:

Beyond Sleep Duration: Distinct Sleep Dimensions are Associated with Obesity in Children and Adolescents

Jarrin, D. C., McGrath, J. J., & Drake, C. L. (Under review)

Abstract

Objective: Short sleep duration is recognized as a significant risk factor in childhood obesity; however, the question as to how sleep contributes to the development of obesity remains largely unknown. A majority of pediatric studies have relied on sleep duration as the exclusive measure of sleep; this insular approach may be misleading given that sleep is a dynamic multidimensional construct that extends beyond sleep duration, including sleep *disturbances* and *patterns*. While these sleep dimensions partly overlap, it is necessary to determine their independent relation with obesity, which in turn, may inform a more comprehensive understanding of putative pathophysiological mechanisms linking sleep and obesity. The aim of the present study was to investigate whether sleep dimensions including sleep duration, disturbances, and patterns were individually associated with obesity, independent of multiple covariates. The second objective was to examine whether sleep disturbances and patterns were independent predictors of obesity, after adjusting for sleep duration. Method: Participants included 242 healthy children and adolescents (M_{age} = 12.66, SD = 2.03; 46.1% females). Anthropometric measures included measured waist and hip circumference, body mass index Z-score and percent body fat. Subjective sleep measures included sleep duration, sleep disturbances, sleep quality, and sleep patterns from youth- and parental-report. Results: Youth with larger adiposity and body composition measures reported poorer sleep quality (β_{avg} = -0.14, p<.01), more sleep disturbances (β_{avg} = 0.12, p<.05), and showed a delayed sleep phase pattern (β_{avg} = 0.13, p<.05), independent of age, sex, pubertal status, physical activity, screen time, and sleep duration. Shorter sleep duration was significantly associated with obesity; however, this link was attenuated after adjustment of covariates. Conclusions:

Results suggest sleep measures beyond duration may more precisely capture influences that drive the negative association between sleep and obesity, and thus, yield more robust associations. As such, future studies are needed to better understand how distinct sleep dimensions confer risk for childhood obesity.

Introduction

Over the past decade, sleep curtailment has been identified as a significant risk factor in the etiology and maintenance of childhood obesity. A solid and consistent association between short sleep duration and obesity, cross-sectionally as well as prospectively, has been reported even after controlling for a number of relevant covariates such as age, sex, and other obesity-related behaviors (e.g., physical activity and snacking; Chen, Beydoun, & Wang, 2008; Nielsen, Danielsen, & Sørensen, 2011). Within pediatric populations, meta-analyses demonstrate a clear pattern suggesting short sleep duration is implicated in the etiology and maintenance of obesity (Chen et al., 2008; Nielson et al., 2011). Despite these generally robust findings, some research suggests the relation between sleep duration and obesity in youth is attenuated after adjustment for covariates (e.g., Calamaro et al., 2010; Knutson, 2005; Storfer-Isser, Patel, Babineau, & Redline, 2012). Alas, the majority of pediatric studies have relied on "sleep duration" as the exclusive measure of sleep. This insular approach may be misleading given that sleep is a dynamic multidimensional construct that extends beyond sleep duration, including sleep *disturbances* and *patterns*.

Sleep duration is derived based on the number of hours slept per night. Measures are used to capture average sleep duration ranging from one night to one month with actual or categorical estimates (e.g., >10hrs, 8-9hrs, 6-7hrs, <6 hrs). Sleep duration reflects factors such as biological and developmental sleep needs as well as contextual or lifestyle demands (e.g., school start times, extracurricular activities). However, length of time spent sleeping is directly influenced by other sleep dimensions. Indeed, sleep dimensions distinct from sleep duration are commonly used as diagnostic criteria for

sleep and arousal disorders (American Sleep Disorders Association, 1990). Sleep disturbances refer to myriad dimensions including sleep fragmentation (e.g., arousals or awakenings), sleep disorders (e.g., sleep apnea, parasomnias), and poor sleep quality. Sleep *patterns* refer to circadian rhythm preference and sleep-wake schedules (e.g., sleep timing). These sleep dimensions may contribute to obesity through their impact on specific pathophysiological mechanisms.

Sleep physiology is composed of two major states (rapid eye movement, REM; non-rapid eye movement, NREM) and a cyclical alternating pattern or architecture. REM is characterized by an increase in heart rate, blood pressure, and respiration level compared to NREM sleep (Pannain & Van Cauter, 2007; van Eekelen, Varkevisser, & Kerkhof, 2003). NREM sleep is subdivided into four stages: stages 1 and 2 (light sleep) and stages 3 and 4 (deep or slow-wave-sleep). Slow wave sleep is characterized by increased parasympathetic and decreased sympathetic activation (i.e., reduced brain activity, heart rate, cardiac output, breathing, and blood pressure compared to wake and REM sleep) and coincides with the most prominent changes in the endocrine system (i.e., stimulating and inhibiting hormone secretion; Hanlon & Van Cauter, 2011; Pannain & Van Cauter, 2007; van Eekelen et al., 2003). Greater time spent in slow wave sleep is considered to be more restorative than other sleep stages given its predominant parasympathetic drive (Edinger et al., 2000; Hanlon & Van Cauter, 2011; Pannain & Van Cauter, 2007; van Eekelen et al., 2003). These sleep dimensions have been linked to adverse physiological processes (Hanlon & Van Cauter, 2011).

Sleep disturbances are largely characterized by recurrent nocturnal awakenings defined by specific EEG events (i.e., micro-arousals) or behavioral markers (e.g.,

reported awakenings; Janackova & Sforza, 2008). Nocturnal awakenings cause abrupt physiological changes markedly increasing sympathetic and hypothalamic pituitary adrenal activity (e.g., increased respiration, heart rate, blood pressure, cortisol; Ekstedt, Åkerstedt, & Söderström, 2004; Janackova & Sforza, 2008; Stamatakis & Punjabi, 2010). In sleep apnea, respiration is repeatedly disrupted, resulting in frequent awakenings and micro-arousals that in turn affect sleep quality. Interestingly, these systems are postulated to contribute to an increased deposit of fat, particularly in the abdominal regions in both adults and youth (Björntorp, 2001; Drapeau, Therrien, Richard, & Tremblay, 2003; Daniels, Morrison, Sprencher, Khoury, & Kimball, 1999).

Sleep patterns related to circadian rhythm preference are biologically governed by the suprachiasmatic nucleus and have a bi-directional relationship with metabolism (Pan & Hussain, 2009; Turek et al., 2005; Laposky, Bass, Kohsaka, & Turek, 2008). Circadian clock mutant mice absorb more carbohydrates and lipids than peptides (Pan & Hussain, 2009), show increased levels of cholesterol, triglycerides, glucose, leptin, and have decreased insulin resistance (Turek et al., 2005; Laposky, Bass, Kohsaka, & Turek, 2008). In turn, metabolic factors feedback onto the regulation of circadian timing, disturbing sleep architecture (time spent in sleep stages), wake schedules, as well as locomotor and feeding behaviors (Turek et al., 2005; Laposky et al., 2008). Taken together, it is speculated that unique sleep dimensions may play critical, yet distinct roles beyond sleep duration (e.g., via pathophysiological mechanisms) in the development and maintenance of obesity.

Among adults, frequent sleep complaints related to initiating and maintaining continuous sleep are significantly associated with greater body mass index (BMI; Strine

& Chapman, 2005; Wheaton et al., 2011) and future weight gain, independent of sleep duration (Lyytikainen, Lallukka, Lahelma, & Rahkonen, 2011). Objectively measured sleep fragmentation (i.e., actigraphy) has been significantly associated with greater BMI (Lauderdale et al., 2009), even in models adjusted for sleep apnea (van den Berg et al., 2008). Further, adults with erratic sleep patterns (e.g., shift work schedules) typically show greater indices of overweight, obesity, and metabolic syndrome compared to those with routine sleep patterns (Di Lorenzo et al., 2003). Among pediatric populations, overweight youth evidence greater sleep disturbances (e.g., sleep-disordered breathing), more arousals (e.g., sleep fragmentation), sleep disorders (e.g., parasomnias), and longer time spent falling asleep (i.e., sleep latency), compared to healthy weight youth (Beebe et al., 2006). Additionally, obese youth report more erratic sleep patterns and later bedtimes, independent of age, sex, and sleep duration (Moore et al., 2011; Olds, Maher, & Matricciani, 2011).

The question as to how sleep contributes to the development of obesity remains largely unknown. The use of sleep duration as the predominant measure of sleep is problematic because it does not reflect the nuanced dimensions underlying sleep which themselves reflect unique physiological processes. In other words, it is unclear whether short sleep duration is directly linked to obesity or whether sleep disturbances (e.g., fragmentation, apnea, quality) and sleep patterns better explain the association. While these sleep dimensions partly overlap, it is necessary to determine their independent relation with obesity, which in turn, may inform a more comprehensive understanding of putative pathophysiological mechanisms linking sleep and obesity.

The aim of the present study was to investigate whether sleep dimensions including sleep duration, sleep disturbances, and sleep patterns were individually associated with obesity in a sample of healthy youth. The second objective was to examine whether sleep disturbances and sleep patterns were independent predictors of obesity, after adjusting for sleep duration. First, it was hypothesized that sleep duration (i.e., school night, weekend night), sleep disturbances (e.g., poor sleep quality), and sleep patterns (e.g., weekend oversleep, weekend delay) would each be significantly associated with greater adiposity (i.e., waist and hip circumference) and body composition indices (i.e., BMI Z-score, percent body fat). Second, it was hypothesized that the associations between sleep disturbances and sleep patterns with obesity would remain significant, even after controlling for sleep duration.

Method

Participants

Youth (N = 242) aged 8 to 18 years and their parents took part in the larger Healthy Heart Project, a longitudinal study that investigates childhood risk factors of cardiovascular disease. Youth were recruited using flyers posted around the community and bookmarks distributed by teachers in classrooms. Exclusionary criteria included serious psychopathology, medical conditions, or use of medications with known cardiovascular effects. The study was approved by the Concordia University Research Ethics Board (# UH2005-077-4). Informed consent and assent were obtained before the start of the study. Participants were financially compensated for their participation time.

Obesity Measures

Anthropometric measures were taken by trained research assistants while youth were dressed in light clothing. Height was measured using a standard stadiometer at maximal breath with shoes off. With a standard measuring tape, waist circumference was measured at the narrowest part of the body, midway between the lowest rib cage and the iliac crest; hip circumference was measured at the widest part of the body over the buttocks. Weight and percent body fat were measured with a bioelectrical impedance scale (Tanita Body Composition Analyzer BF-350). Bioelectrical impedance methods have demonstrated moderate agreement with the gold standard for measuring body fat (dual energy x-ray absorbtiometryl method: $r = 0.40$ to 0.69; Pateyjohns, Brinkworth, Buckley, Noakes, & Clifton, 2006). Age-and sex-specific BMI Z-scores were determined using the growth charts published by the U.S. Centers for Disease Control and Prevention (Ogden et al., 2002).

Sleep Measures

Sleep Duration. Sleep duration was obtained by youth self-report in response to: "During the past month, what time do you usually go to bed/wake up on school nights/weekends?" Sleep duration for school nights and weekends was calculated as the difference between bed- and wake-time. Youth self-report estimates of sleep duration have been previously shown to be correlated with objective measures of sleep duration (actigraphy: $r = 0.53$; Wolfson et al., 2003).

Sleep Disturbances. The Children's Sleep Habits Questionnaire is a 43-item scale that screens for common sleep problems (e.g., parasomnias, sleep-disordered breathing) over a one week interval (Owens, Spirito, & McGuinn, 2000). On a 3-pt scale

(rarely, sometimes, usually), parents reported the frequency of their child's sleep habits (e.g., "my child awakes more than once"). Items are summed to obtain a total score, with higher scores indicating considerable sleep disturbances. The scale has demonstrated test-retest reliability, validity, and internal consistency (Owens et al., 2000). Youth also rated their overall sleep quality (i.e., the subjective perception of how sleep is experienced) on a scale of 1 to 10 (1 = very bad to 10 = very good). This question is commonly used in studies assessing explicit perceptions about feeling rested and satisfied with sleep upon awakening (Dewald, Meijer, Oort, Kerkhof, & Bo, 2010).

Sleep Patterns. Sleep patterns were measured with self-reported bed- and wake-time during school and weekends. These times were used to derive weekend oversleep (difference total sleep duration on weekends and school nights), weekend delay (difference between bedtime on weekend and school nights), and weekend awakening delay (difference between waketime on weekend and school days; Smith, Reilly, & Midkiff, 1989). Greater differences indicate more sleep, later bedtimes, and later wake times on weekends, respectively. Self-reported bed- and wake-times are significantly correlated with objective measures of sleep (actigraphy: $r = 0.70$) and wake-onset times (actigraphy: $r = 0.77$; Wolfson et al., 2003).

Covariates

Puberty. Using a validated self-report measure of puberty (Growing and Changing Questionnaire; Golding, Pembrey, & Jones, 2001), two stages of pubertal development were assessed: gonadarche (breast and genital development) and adrenarche (pubic hair). Youth indicated their pubertal stage based on sex-specific illustrations corresponding to Tanner stages I-V of prepubertal to complete sexual maturity. Although

physician assessment of pubertal development is considered the gold standard, self-report has demonstrated good reliability and validity among youth (r= 0.77 to 0.91; Morris & Urdy, 1980).

Locomotor Activity. Youth answered questions about moderate physical activity (e.g., "During the past week, how many days did you do physical activity for 20 min straight, that made you sweat or breathe fast?") and screen time (e.g., "During the past week, how many hours did you watch TV each day?"). Self-report estimates of weekly physical activity bouts in youth yield adequate reliability with accelerometer (r = 0.31) and activity logs (r = 0.46; Welk et al., 2007). Self-report estimates of screen time also show high test-retest reliability among youth (*ICC* = 0.98; He, Harris, Piche, & Beynon, 2009).

Statistical Analysis

All data were analyzed with SPSS 20 software (SPSS, Inc., Chicago, IL), kept continuous to maximize statistical power, and were checked for normality and linearity. To examine the extent of overlap among the sleep dimensions, partial correlational analyses controlling for age were conducted. Next, to test the hypotheses, sequential regression analyses were modeled individually for each obesity measure (i.e., waist circumference, hip circumference, BMI Z-score, percent body fat). First, each sleep dimension (i.e., sleep duration, sleep disturbances, sleep quality, bed- and wake-time, weekend oversleep, weekend delay, weekend awakening delay) was entered singularly to examine its unique effect on each obesity measure. Second, covariates were entered into these models (age, sex, pubertal status, physical activity, screen time). Third, sleep

duration was added as an additional covariate to test the association between each sleep dimension with obesity, beyond sleep duration.

Results

Participant demographics are presented in Table 1. The majority of youth were male (54%), Caucasian (59%), and of normal weight status (70% BMI <85th percentile). On average, youth were 12.66 years (*SD* = 2.03), reported being in the intermediate stages of pubertal adrenarche (*M* = 2.98, *SD* = 1.49), were moderately physically active for ~4 days/week (*SD* = 1.97), and watched TV ~2.5 hours/day (*SD* = 1.94). Youth typically reported their sleep quality as average (range 1-10, *M* = 6.76, *SD* = 2.07), slept ~9 hours on school nights, ~10 hours on weekend nights, and had a later bed- (*M* = 80 min, *SD* = 66) and wake-time (*M* =134 min, *SD* = 100) on weekends (see Table 2).

After controlling for age, partial correlational analyses revealed small to moderate inter-correlations among the sleep dimensions (Table 3). Sleep duration on school and weekend nights was weakly correlated with sleep disturbances and weekend delay. Moderate to large correlations were observed for weekend oversleep and weekend awakening delay for weekend sleep duration only. However, these higher correlations are largely attributable to use of bed- and wake times to derive both of these measures. Sleep disturbances and sleep patterns also yielded low correlations. Collectively, these data suggest the sleep dimensions were largely unique.

In the first step of the sequential regression models, each sleep dimension was entered singularly (see Table 4). School night sleep duration significantly predicted waist and hip circumference. Sleep disturbances significantly predicted waist circumference and BMI, while sleep quality predicted all obesity measures. Sleep patterns were

associated with waist and hip circumference and BMI, but not percent body fat. These findings are largely consistent with previously reported findings.

Second, when covariates (age, sex, pubertal status, physical activity, screen time) were entered into the models (see Table 5), sleep duration was no longer associated with any obesity measures. Sleep disturbances significantly predicted all obesity measures. Sleep patterns reflected by overall bedtimes were most predictive of obesity measures, while indicators of weekend sleep debt (e.g., oversleep, delay) were not related.

Third, when sleep duration was added as another covariate, analyses revealed nearly largely identical results (data not shown for parsimony). Namely, sleep disturbances remained significantly associated with adiposity and body composition indices. Similarly, sleep patterns of later school night bed- and wake-times and later weekend bedtimes were still significantly associated with adiposity and body composition measures.

Discussion

The relation between sleep and obesity has been predominantly limited to the use of sleep duration. However, sleep duration is a broad measure that does not capture the unique aspects of other sleep dimensions. Importantly, different sleep dimensions may provide more precise information to better elucidate the relation between sleep and obesity due to their distinct underlying physiological mechanisms. The aim of the present study was to assess whether obesity measures are better predicted by sleep disturbances and sleep patterns, beyond sleep duration.

Consistent with past research, school night sleep duration was significantly associated with central adiposity measures of obesity in youth (Chen et al., 2008; Nielson

et al., 2011), such that short sleep duration predicted greater waist and hip circumference. However, this association was attenuated after the adjustment of multiple covariates; similar results have also been previously reported (Calamaro et al., 2010; Knutson, 2005; Storfer-Isser et al., 2012). Sleep disturbances, on the other hand remained significantly associated with obesity in both unadjusted and adjusted models. Youth exhibiting frequent sleep disturbances had larger waist and hip circumferences as well as greater percent body fat. Beebe et al (2006) found greater parent-reports of parasomnias, daytime sleepiness, and bedtime resistance among clinically obese youth compared to healthy weight controls. In the present sample, pre-sleep anxiety and bedtime resistance were significant predictors of obesity indicators (data not shown). Similarly, frequent childhood sleep disturbances were associated with an almost two-fold increased risk of being overweight or obese at age 21 (Al Mamum et al., 2002). Childhood sleep problems (e.g., pre-sleep anxiety), if untreated, may evolve into eventual sleep disorders (e.g., insomnia; Moore, Allison, & Rosen, 2006). Notably, there is physiological evidence showing markedly reduced parasympathetic and increased sympathetic activation during the day and night among children diagnosed with sleep disorders, such as sleep-disordered breathing (Liao et al., 2010) and periodic leg movements (Walter et al., 2009).

Consistent with past studies, sleep quality was significantly associated with obesity (Bawazeer et al., 2009). Youth reporting poor sleep quality had larger hip circumference, BMI Z-score, and percent body fat, after controlling for covariates. Poor sleep quality has been linked with an increased likelihood of having high blood pressure among adolescents (Javaheri, Storfer-Isser, Rosen, & Redline, 2008) and greater waist circumference, BMI, percent body fat, insulin and glucose concentrations, and insulin

resistance among adults (Jennings, Muldoon, Hall, Buysse, & Manuck, 2007). It has been postulated that estimates of sleep quality may be an indirect marker of restorative slow wave sleep (Edinger et al., 2000). Compared to other sleep stages, slow wave sleep is particularly relevant for metabolic, hormonal, and neurophysiologic homeostasis (Hanlon & Van Cauter, 2011; van Eekelen et al., 2003). During slow wave sleep, there is an overall dominance of parasympathetic activity and concomitant reductions in sympathetic activity, glucose use, and corticotropic release (i.e., cortisol and ACTH; Hanlon & Van Cauter, 2011; van Eekelen et al., 2003), and is thus, posited to be an especially important sleep stage to obtain.

Experimental evidence indicates that selective deprivation of slow wave sleep may contribute to poor metabolism via the dysregulation of insulin, increase in cortisol secretion, and reduced secretion of growth hormone (Tasali, Leproult, Ehrmann, & Van Cauter, 2008). Less time in slow wave sleep was associated with greater BMI, waist and hip circumference, percent body fat, and waist-to-hip ratio after controlling for physical activity, sleep efficiency, snoring, and sleep duration among older men (Rao et al., 2009) and middle-aged women (Theorell-Haglöw, Berne, Janson, Sahlin, & Lindberg, 2010).

Sleep patterns also emerged as a significant predictor of obesity. Youth reporting later weekend bedtimes and exhibiting a delayed sleep phase on school days (i.e., later sleep and wake times) had greater adiposity and body composition measures of obesity, regardless of sleep duration. This is consistent with evidence that metabolism may be more influenced by the *timing* of sleep (i.e., obtaining sleep at one's natural/ideal point in their circadian rhythm), rather than the actual *quantity* of sleep obtained (Scheer, Hilton, Mantzoros, & Shea, 2009). Although the present study found no significant association

between irregular weekend sleep schedules and obesity in youth after adjusting for covariates, the observed direction is similar to those previously reported (Olds, Tomkinson, Maher, & Ferrar, 2008). Collectively, youth who exhibit more sleep disturbances, perceive their sleep quality as poor, and have a delayed sleep phase show significantly greater adiposity and body composition indices of obesity, irrespective of sleep duration.

Potential Underlying Mechanisms

The parasympathetic nervous system is dominant during sleep. Nocturnal awakenings or the transition to wakefulness are associated with concomitant increases in sympathetic (i.e., norepinephrine, sympathetic muscle nerve activity, blood pressure, heart rate) and hypothalamic pituitary adrenal activity (i.e., cortisol; Hanlon & Van Cauter, 2011; van Eekelen et al., 2003). Recurrent nocturnal awakenings considerably reduce sleep quality, increase daytime sleepiness, and alter sleep architecture, reducing restorative slow wave sleep and REM sleep (Ekstedt et al., 2004; Stamatakis & Punjabi, 2010). This reduction in slow wave sleep leads to decreases in growth hormone secretion, insulin sensitivity, and glucose effectiveness, as well as increases in morning cortisol and cholesterol levels; all irrespective of sleep duration (Ekstedt et al., 2004; Stamatakis & Punjabi, 2010). Additionally, sleep disruptions (sleep fragmentation) may modify the ability of appetite-regulating hormones (e.g., glucose, leptin, ghrelin) to accurately signal appropriate energy intake and expenditure, leading to increased food consumption, particularly for unhealthy foods (i.e., high-carbohydrate foods) and weight gain (Pannain & Van Cauter, 2008). It is postulated that the integrated activation of these systems contributes to increased metabolism of specific lipid-accumulating key enzymes,

targeting lipolytically sensitive adipose tissue regions (Björntorp , 2001; Drapeau et al., 1999). Thus, the pathophysiological responses related to shallow fragmented sleep may promote a nocturnal stress response within the nervous and endocrine system, expediting the progression of obesity; examples of this include data from those diagnosed with sleep apnea (Trakada, Chrousos, Pejovic, & Vgontzas, 2007).

Another plausible mechanism underlying the relation between sleep and obesity is the neurotransmitter hypocretin (Hcrt), also referred to as orexin. Hcrt is related to stress induced wakefulness, which leads to a hyperarousal state commonly characterized by increased fatigue, anxiety, and insomnia (Baumann & Bassetti, 2005). It is also implicated in autonomic functions (i.e., increased arterial blood pressure, heart rate, and overall sympathetic activation) and has direct effects on the regulation of sleep-wake behaviors (Baumann & Bassetti, 2005). Interestingly, Hcrt activation may also promote increased food intake, particularly palatable food, via activation of appetite regulating neurons (e.g., neuropeptide Y and agouti-related peptide) and inhibition of appetite suppressing neurons (e.g., proopiomelanocortin and cocaine-and amphetamine-related transcripts; Sakurai, 2007; vandenTop, Lee, Whyment, Blanks, & Spanswick, 2004; Zheng, Patterson, & Berthoud, 2005). Taken together, the integrated activation of the sympathetic and hypothalamic pituitary adrenal systems in combination with the Hcrt system are putative physiological mechanisms underlying the association between sleep disturbance and obesity (Hanlon & Van Cauter, 2011).

Strengths and Limitations

One limitation of the current study was the cross-sectional design; the present findings cannot determine the temporal direction of the relation between sleep and

obesity. A second limitation involved the subjective measures of sleep indices, which precluded diagnosis of sleep disorders, such as sleep apnea or restless leg syndrome. Although, the subjective measures used in the present study are likely to be more related to habitual sleep duration than a single laboratory measure; and they have demonstrated reliability and validity in the literature when compared with objective sleep measures (Lockley, Skene, Butler, & Arendt, 1999). Further, unlike previous studies, multiple indicators of obesity and sleep were measured. Sleep parameters were assessed by multiple informants, which provide a more comprehensive representation of sleep complaints, sleep patterns on both school and weekends, as well as explicit ratings of sleep quality by youth. This was corroborated when sleep demographics (e.g., average sleep duration) within our sample were similar with those reported in past pediatric research (Moore et al., 2011). Similarly, the sample was representative of the general population, with prevalence rates of healthy-weight, overweight, and obese youth similar to population-based studies (Tremblay, Katzmarzyk, & Willms, 2002). This multi-method approach likely provides more robust measures of obesity and sleep in youth.

Future Studies and Conclusions

Given that obesity is a risk factor for multiple chronic diseases (e.g., cardiovascular disease), disability, and premature mortality (Must & Anderson, 2000), a better understanding of the role of sleep in the pathogenesis of obesity is of great importance. Longitudinal designs with repeated obesity and sleep measures are necessary to elucidate how weight gain is influenced by distinct sleep dimensions (i.e., sleep disturbances and patterns). Specifically, investigation of the role of chronobiological (e.g., evening/morning preference), physiological (e.g., autonomic and

metabolic activity profiles across specific sleep stages), contextual (e.g., school start-times, parental monitoring), and psychological (e.g., stress) factors across the life course is recommended to better delineate the nature and direction of the obesity-sleep relation (Hale & Berger, 2011). It may be advantageous to consider using ambulatory polysomnography, actigraphy, and daily sleep logs completed in the participant's usual environment, which would provide both objective and subjective information on sleep schedules (e.g., during school vs. summer vacations) and habits (e.g., watching TV in bed), as well as potentially identify additional risk and protective factors (e.g., sleep duration thresholds) previously omitted in studies (Hale & Berger, 2011). Given the dynamic nature of sleep and obesity during childhood, future research has the potential to identify important obesity prevention strategies such as lifestyle habits (e.g., sleep hygiene) that develop during the particularly vulnerable childhood life stage.

Overall, consistent with the literature, short sleep duration was associated with childhood obesity. The results suggest sleep measures beyond duration may more precisely capture influences that drive the negative association between sleep and obesity, and thus, yield more robust associations. Sleep disturbances and sleep delayed phase sleep pattern were independently associated with greater adiposity and body composition indices of obesity in youth, irrespective of obesity-related covariates and sleep duration.

Acknowledgements: Special thanks to the participants of the Healthy Heart Project, the Pediatric Public Health Psychology Laboratory research assistants and study coordinators, Natasha Hunt and Sabrina Giovanniello. The data used in these analyses were drawn from the Healthy Heart Project. This work was made possible through funding support from the Canadian Institute of Health Research (CIHR; MOP89886; OCO79897) and the Fonds de la recherche en santé du Québec (FRSQ; 16965).

Table 1

Demographic Information of Youth

Variable	*Mean (n)*	*SD (%)*
Sex		
Girls	(110)	(46.1)
Age (years)	12.66	2.03
Race		
Caucasian	(145)	(59.7)
Asian	(26)	(10.7)
Black	(20)	(8.2)
Latino	(10)	(4.1)
Other/Mixed	(42)	(17.3)
Anthropometric Measures		
Waist circumference (cm)	72.12	9.17
Hip circumference (cm)	90.7	10.48
Body Mass Index (%^{percentile})	63.27	26.66
Percent body fat (%)	21.95	9.23
Body weight status[1]		
Normal (5^{th} - $<85^{th\ percentile}$)	(172)	(70.8)
Overweight (85^{th} - $95^{th\ percentile}$)	(43)	(17.8)
Obesity ($\geq 95^{th\ percentile}$)	(25)	(10.3)
Underweight ($<5^{th\ percentile}$)	(3)	(1.2)
Pubertal Stage (adrenarche)	2.98	1.49
Physical Activity (days/week)	3.72	1.97
Screen time (hrs/day)	1.86	1.94

Note. N = 242.

[1]Distribution of age-and sex-specific body weight based on Centers for Disease Control values

Table 2

Demographic Measures of Sleep Dimensions in Youth

Sleep Dimensions	*Mean*	*SD*
Sleep Duration		
School night (min)	545.11	62.96
Weekend night (min)	598.69	82.78
Sleep Disturbances[1]		
Sleep Behavior Problems[1] (0-132)	41.27	5.21
Bedtime Resistance (range 5-15)	6.95	1.45
Sleep Onset Latency (range 1-3)	1.31	0.57
Sleep Duration (range 3-9)	4.31	1.54
Sleep Anxiety (range 3-9)	4.45	0.88
Night Awakenings (range 3-6)	3.29	0.65
Parasomnias (range 6-17)	7.65	1.14
Sleep-Disordered Breathing (range 3-5)	3.14	0.42
Sleep Quality (1-10)	6.76	2.07
Sleep Patterns		
School Night		
Wake time (hr:min)	6:51	00:35
Bedtime (hr:min)	21:47	00:53
Weekend Night		
Wake time (hr:min)	9:06	1:34
Bedtime (hr:min)	23:07	1:22
Weekend Oversleep (min)	53	95
Weekend Delay (min)	80	66
Weekend Awakening (min)	134	100

Note. N = 242. (hr : min = hour : minutes). [1]Children's Sleep Habits Questionnaire.

Table 3

Partial Correlations among Sleep Dimensions, Controlling for Age

	Sleep Duration		Sleep Disturbances		Sleep Patterns		
	School Night	Weekend Night	Sleep Disturbances	Sleep Quality	Weekend Oversleep	Weekend Delay	Weekend Awakening Delay
School Night	-	**0.23*****	**-0.15****	**0.18****	**-0.35*****	-0.01	-0.10
Weekend Night		-	**-0.17****	-0.01	**0.82*****	**-0.18****	**0.27*****
Sleep Disturbances			-	**-0.20****	-0.07	0.05	-0.06
Sleep Quality				-	-0.12	-0.00	-0.08
Weekend Oversleep					-	**-0.16****	**0.32*****
Weekend Delay						-	**0.38*****
Weekend Awakening Delay							-

$^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$

Table 4

Beta Regression Coefficients of Sleep Dimensions Entered Singularly

	Waist Circumference			Hip Circumference			Body Mass Index[1]			Percent Body Fat		
	β	*t*	*p*	*β*	*t*	*p*	*β*	*t*	*p*	*β*	*t*	*p*
Sleep Duration												
School Night	**-0.22**	**-3.47**	**0.00**	**-0.40**	**-6.78**	**0.00**	0.02	0.31	0.75	-0.05	-0.78	0.43
Weekend Night	-0.04	-0.73	0.46	-0.01	-0.15	0.87	-0.08	-1.28	0.19	-0.01	-0.20	0.84
Sleep Disturbances												
Sleep Disturbances[2]	**0.15**	**2.41**	**0.01**	0.11	1.81	0.07	**0.13**	**2.00**	**0.04**	0.10	1.67	0.09
Sleep Quality	**-0.16**	**-2.60**	**0.01**	**-0.27**	**-4.43**	**0.00**	**-0.14**	**-2.23**	**0.02**	**-0.16**	**-2.51**	**0.01**
Sleep Patterns												
School Bedtime	**0.23**	**3.81**	**0.00**	**0.31**	**5.19**	**0.00**	0.10	1.62	0.10	0.07	1.23	0.22
School Wake time	0.05	0.86	0.38	-0.05	-0.90	0.36	**0.15**	**2.44**	**0.01**	0.10	1.62	0.10
Weekend Bedtime	**0.24**	**3.90**	**0.00**	**0.28**	**4.61**	**0.00**	**0.18**	**2.91**	**0.00**	0.07	1.22	0.22
Weekend Wake time	**0.14**	**2.20**	**0.02**	**0.21**	**3.34**	**0.00**	0.03	0.54	0.58	0.02	0.41	0.68
Weekend Oversleep	0.10	1.60	0.10	**0.25**	**4.09**	**0.00**	-0.08	-1.32	0.18	0.02	0.34	0.73
Weekend Delay	0.11	1.75	0.08	0.10	1.57	0.11	**0.14**	**2.28**	**0.02**	0.03	0.54	0.58
Weekend Awakening Delay	0.11	1.75	0.08	**0.21**	**3.46**	**0.00**	-0.02	-0.34	0.73	-0.01	-0.19	0.84

Note. $N = 242$. [1]Body mass index in Z-score. [2]Children's Sleep Habits Questionnaire. β = standardized regression coefficient. This model includes each sleep dimension entered singularly without covariates. Bolded values indicate statistical significance.

Table 5

Beta Regression Coefficients of Sleep Dimensions Entered Simultaneously with Covariates

	Waist Circumference			Hip Circumference			Body Mass Index[1]			Percent Body Fat		
	β	*t*	*p*	*β*	*t*	*p*	*β*	*t*	*p*	*β*	*t*	*p*
Sleep Duration												
School Night	-0.03	-0.38	0.70	-0.09	-1.42	0.15	-0.01	-0.13	0.89	0.02	0.29	0.76
Weekend Night	-0.05	-0.81	0.41	-0.04	-0.68	0.49	-0.07	-1.31	0.19	-0.08	-1.31	0.18
Sleep Disturbances												
Sleep Disturbances[2]	**0.15**	**2.56**	**0.01**	**0.10**	**1.93**	**0.05**	0.12	1.76	0.07	**0.11**	**1.98**	**0.04**
Sleep Quality	-0.11	-1.71	0.08	**-0.15**	**-2.85**	**0.00**	**-0.16**	**-2.42**	**0.01**	**-0.12**	**-1.98**	**0.04**
Sleep Patterns												
School Bedtime	0.09	1.31	0.19	0.09	1.57	0.11	**0.14**	**1.98**	**0.04**	**0.12**	**1.96**	**0.05**
School Wake time	**0.13**	**2.07**	**0.03**	0.05	0.90	0.36	**0.17**	**2.63**	**0.00**	**0.15**	**2.68**	**0.00**
Weekend Bedtime	**0.13**	**2.02**	**0.04**	**0.13**	**2.28**	**0.02**	**0.19**	**2.79**	**0.00**	0.11	1.85	0.06
Weekend Wake time	0.05	0.83	0.40	0.08	1.46	0.14	0.04	0.53	0.59	-0.00	-0.02	0.98
Weekend Oversleep	-0.04	-0.55	0.57	0.01	0.18	0.85	-0.09	-1.19	0.23	-0.09	-1.45	0.14
Weekend Delay	0.09	1.44	0.15	0.08	1.55	0.12	0.12	1.84	0.06	0.04	0.76	0.44
Weekend Awakening Delay	0.00	0.05	0.95	0.06	1.07	0.28	-0.03	-0.42	0.67	-0.07	-1.17	0.24

Note. $N = 242$. [1]Body mass index in Z-score. [2]Children's Sleep Habits Questionnaire. β = standardized regression coefficient. Multivariate models include each sleep variable entered simultaneously with covariates (age, sex, pubertal status, physical activity, and screen time). Results remained consistent while adjusting for sleep duration (data not shown). Bolded values indicate statistical significance.

TRANSITION TO MANUSCRIPT 4

Given that sleep is dynamic and comprised of numerous dimensions, including duration, patterns, and disturbances, the aim of manuscript 3 was to investigate whether sleep parameters beyond sleep duration were associated with obesity indices among healthy children and adolescents. The novel findings suggest that other sleep dimensions may more precisely capture the influences that drive the negative association between sleep and childhood obesity. Youth with larger central adiposity and body composition measures reported poorer sleep quality, more sleep disturbances, and evidenced a delayed sleep phase pattern, independent of sleep duration. Surprisingly, sleep duration was associated with childhood obesity in unadjusted, but not adjusted models.

These findings about the independent relation of multiple sleep dimensions with child obesity informed my conceptual framework for understanding the underlying physiological mechanisms mediating the link between sleep and obesity. My conceptualization broadened to consider whether sympathovagal imbalance could potentially underlie the relation between obesity with, not only short sleep duration, but with other sleep dimensions that may have their own distinct pathophysiological influences on the etiology and maintenance of obesity. Interestingly, sympathovagal imbalance has been proposed to be involved with distinct sleep dimensions, both directly and indirectly (Knutson, 2012; Spiegel et al., 1999, 2004).

The objective of manuscript 4 was to assess whether sympathovagal imbalance mediated the relation between sleep and obesity in youth. This fourth manuscript was directly informed by the culmination of my earlier work. Manuscript 1 informed the selection of variables for testing the posited sympathovagal balance mechanism.

Namely, sympathovagal imbalance was assessed using frequency-domain variables: high-frequency (HF), low-frequency (LF) and LF:HF ratio. These HRV parameters were found to have excellent measurement fidelity across commonly used software programs. Based on my results as well as previous findings in the literature, VLF was not used due to its ambiguous interpretation and moderate measurement fidelity with other software programs. It was decided to focus exclusively on these frequency-domain variables as they are considered more sensitive indicators of parasympathetic and sympathetic activity (Task Force, 1996) and because they are commonly assessed and reported within the sleep literature (Speigel et al., 1999, 2004; Van Cauter, 2008; Zhong et al., 2005).

Manuscript 2 informed the selection of developmentally-relevant covariates that were controlled for methodologically (e.g., consistent ECG start time) and statistically (e.g., heart rate, puberty, blood pressue). The normative HRV values were used to compare the results obtained with a sample at-risk for obesity. Manuscript 3 informed the selection of sleep dimensions to be tested, including sleep duration, sleep patterns, and sleep disturbances. Collectively, my previous findings directly informed and enabled examination of my overarching research question. The aim of manuscript 4 was to assess whether sympathovagal imbalance mediated the link between multiple sleep parameters and childhood obesity.

MANUSCRIPT 4:

Sympathovagal Imbalance Mediates the Relation between Sleep and Obesity in Children

Jarrin, D. C., McGrath, J. J., Poirier, P., & Paradis, G. (Submitted.)

Abstract

While mounting evidence suggests sleep plays a causal role in the development of obesity, the underlying pathogenic pathways are complex and unresolved. Experimental sleep deprivation studies demonstrate sympathovagal imbalance (i.e., hyperactive sympathetic and or hypoactive parasympathetic activity) is consequent to inadequate sleep. Further, obese children exhibit sympathovagal imbalance, particularly during the night, compared to non-obese children. The question remains whether sympathovagal imbalance is one potential pathophysiological pathway underlying the association between sleep and obesity. The aim of the present study was to examine whether sympathovagal imbalance mediated the relation between sleep and childhood obesity. Participants included 564 children aged 9 to 12 years (43.6% girls) from QUALITY, a longitudinal cohort study of youth at-risk for the development of obesity. Sleep duration, patterns, and disturbances were based on child- and parent-report. Anthropometrics were measured for central adiposity and body composition indices of obesity (e.g., waist and hip circumference, percent body fat, fat mass index). Sympathovagal imbalance was derived from heart rate variability spectral analyses of an electrocardiograph recording. Sympathovagal imbalance partially mediated the relation between inadequate sleep with central adiposity (R^2_{avg} = 0.25, ΔR^2_{avg} = 0.02) and body composition (R^2_{avg} = 0.14, ΔR^2_{avg} = 0.01). Future research should consider longitudinal designs with additional physiological measures of sympathovagal imbalance (e.g., coherence analysis, cardiopulmonary coupling) acquired during sleep. These findings highlight the importance of better understanding sympathovagal imbalance and its role in the etiology and maintenance of obesity.

Introduction

Childhood obesity is a global epidemic with increasing prevalence rates over the past two decades (Shields, 2006; Tremblay, Katzmarzyk, & Willms, 2002). Although recent data suggest the prevalence may have plateaued, rates of childhood obesity are high and contribute to significant physical, psychological, and economic burden (Ogdon, Carroll, Curtin, Lamb, & Flegal, 2010; Ogdon, Carroll, Kit, & Flegal, 2012). Notably, childhood obesity tracks into adulthood (Freedman, Khan, Serdula, Dietz, Srinivasan & Berernson, 2005) and confers risk for insulin resistance, impaired glucose tolerance, hypertension, and early precursors to cardiovascular disease (Freedman, Dietz, Srinivasan, & Berenson, 1999; Must & Strauss, 1999; Wang & Dietz, 2002).

Mirroring these trends, shorter sleep duration has increased among youth, largely due to later bedtimes and unchanged rise times across these past decades (Iglowstein, Jenni, Molinari, & Largo, 2003). Only 20% of youth report sleeping the recommended 9 hours each night and more than half of adolescents report sleeping 1 to 2 hours less than recommended (Nathional Sleep Foundation [NSF] Survey, 2004, 2008). Further, worldwide studies estimate 20% to 30% of children and 6% to 37% of adolescents (Liu et al., 2000; NSF Survey, 2004, 2008) report problems related to prolonged sleep latency, difficulty initiating and maintaining sleep, frequent nocturnal awakenings, and poor quality sleep accompanied with significant daytime impairments (Archold, Pituch, Panabi, & Chevrin, 2002; Owens, Spirito, & McGuinn, 2000; Roberts, Roberts, & Chan, 2006).

Numerous cross-sectional studies report an inverse association between shorter sleep duration, poor sleep quality, sleep disturbances (e.g., parasomnias, nocturnal awakenings), and a delayed sleep phase pattern with larger body composition (e.g., body

mass index [BMI], percent body fat), greater central adiposity (e.g., waist, hip circumference), and increased obesity rates among both adults and youth (c.f., Beebe et al., 2006; Chaput, Brunet, & Tremblay, 2006; Chaput & Tremblay, 2007; Nielsen, Danielsen, & Sørensen, 2011; Jarrin, McGrath, & Drake, 2011; Knutson & Van Cauter, 2008; Liu et al., 2011; Marshall, Glozier, & Grunstein, 2008). Longitudinal studies also support the causal role inadequate sleep (i.e., short sleep duration and sleep disturbances) has on the development of obesity (c.f., Carter, Taylor, Williams, & Taylor, 2011; Snell, Adam, & Duncan, 2006; Taveras, Rifas-Shiman, Oken, Gunderson, & Gillman, 2008); however, the mechanisms underlying this association are still not fully understood.

Experimental sleep deprivation studies with adults provide valuable insight into potential mechanistic pathways linking sleep and obesity. One plausible pathophysiological mechanism is the modulation of cardiac autonomic dysfunction, reflected by *sympathovagal imbalance* (i.e., hyperactive sympathetic nervous system and or a hypoactive parasympathetic nervous system; Hanlon & Van Cauter, 2011; Knutson, Spiegel, Penev, & Van Cauter, 2007; Van Cauter et al., 2007). Heart rate variability (HRV) is commonly derived from spectral analysis (e.g., Fast Fourier transform [FFT]), as a measure of sympathovagal imbalance. HRV reflects beat-to-beat (RR) variation in heart rate, which is influenced by the combined effects of both sympathetic and parasympathetic activity on the sino-atrial node. Spectral analysis transforms heart rate from RR intervals to power bands that reflect indices of sympathetic and parasympathetic modulation in the cardiac system (Lahiri, Prince, Kannankeril, & Goldberger, 2008; Spiers, Silke, McDermott, Shanks, & Harron, 1993; Task Force, 1996). Low frequency power band (LF = 0.04–0.15 Hz) reflects the aggregate influences of parasympathetic and

sympathetic branches of the autonomic nervous system (Akselrod et al., 1981; Berntson et al., 1997; Task Force, 1996). High frequency power band (HF = 0.15–0.40 Hz) represents parasympathetic activity (Berntson et al., 1997; Pomeranz et al., 1985; Task Force, 1996). Sympathovagal imbalance is denoted by higher LF:HF ratio (Malliani, Pagani, & Lombardi, 1994; Montano, Tobaldini, & Porta, 2012) and based on microneurography and ganglionlic blockade studies, is recognized as an appropriate measure of sympathetic modulation (Diedrich et al., 2003; Pagani et al., 1997). Further, sympathovagal imbalance has been consistently used as a marker of autonomic dysfuction (c.f., Hall et al., 2004; Malliani et al., 1994; Spiegel, Leproult, & Van Cauter, 1999; Spiegel et al., 2004).

Several studies have documented sympathovagal imbalance following sleep deprivation. Sgoifo and colleagues (2006) deprived rats of sleep for 48 hours by placing them on a slowly rotating wheel. Compared to controls, the sleep deprived rats showed significantly decreased parasympathetic activity (i.e., low HRV) during dark and light phases, which endured even after two days under sleep recovery conditions. Among healthy adults, sympathovagal imbalance significantly increased 20% after partial sleep deprivation (LF:HF ratio 1.81 to 2.17; Zhong et al., 2005) and 15% after total sleep deprivation (LF:HF ratio 2.66 to 3.06; Tochikubo, Ikeda, Miyajima, & Ishii, 1996).

Likewise, parasympathetic modulation (HF) significantly decreased 19% after partial sleep deprivation (Tochikubo et al., 1996) and 22% after total sleep deprivation (Zhong et al., 2005). Under partial sleep deprivation over 6 nights (e.g., 4 hour sleep), healthy males evidenced significant increases in sympathovagal imbalance as compared to conditions of sleep recovery (Spiegel et al., 1999, 2004). The increases in

sympathovagal imbalance were particularly evident the following morning (9 a.m.-1 p.m.) and afternoon (1-5 p.m.) with 16% to 19% increases, respectively. Preliminary work in children has yielded parallel results. Sleep deprived infants (i.e., no napping) had LF:HF ratio twice as high compared to those in the napping condition (LF:HF ratio 3.12 *vs*. 1.57; Franco et al., 2003). Children aged 9 years who objectively slept one hour less evidenced lower parasympathetic and significantly higher sympathovagal imbalance, compared to those with an additional hour of sleep (Rodriguez-Colon et al., 2011). Taken together, findings across adults and children suggest that sympathovagal imbalance occurs as a function of sleep loss.

The relation between sympathovagal imbalance and sleep is further supported by research examining sleep disturbances. Among adults, sympathovagal imbalance is significantly associated with sleep disorders (e.g., insomnia, sleep apnea; Bonnet & Arand, 2010; Somers, Dyken, Clary, & Abboud, 1995), sleep fragmentation (Stamatakis & Punjabi, 2010), and subjective (Burton, Rahman, Kadota, Lloyd, & Vollmer-Conna, 2010; Wei, Chung, Wu, Chung, & Wu, 2011) and objective reports of poor sleep quality (Tasali, Leproult, Ehrmann, & Van Cauter, 2008). For example, sympathovagal imbalance, indexed by LF:HF ratio, significantly increased by 37% and parasympathetic activation, indexed by HF, significantly decreased by 14% after three consecutive nights of poor sleep quality (i.e., slow wave sleep suppression) among adults, even after controlling for breathing frequency, total sleep time, and total wake time (Tasali et al., 2008).

Among children, those with obstructive sleep apnea evidence a 9% increase in sympathovagal imbalance, a 22% reduction in overall HRV (i.e., LF), and a 25%

reduction in parasympathetic activity the morning following an overnight polysomnography, compared to healthy controls (Kwok et al., 2011). Similarly, children with sleep fragmentation, such as periodic leg movements (Walter et al., 2009) and sleep-disordered breathing symptoms, exhibit enhanced sympathetic and weaker parasympathetic modulation (Liao et al., 2010). Specifically, children with moderate sleep-disordered breathing symptoms had significantly lower HF and significantly greater LF:HF ratio values, compared to children with none or mild symptoms (Liao et al., 2010). Children with insomnia symptoms (e.g., difficulty initiating and maintaining sleep) during the past two months had reduced parasympathetic modulation and increased sympathovagal imbalance, even after adjusting for multiple covariates (Rodriguez-Colon et al., 2011). Taken together, evidence indicates inadequate sleep is associated with sympathovagal imbalance (i.e., sympathetic hyperactivity and reduced parasympathetic activation).

Sympathovagal imbalance is considered an important risk factor in the development of cardiovascular diseases (e.g., coronary heart disease), diabetes, insulin resistance, hypertension, and obesity (Knutson, 2012; Palatini & Julius, 1997; Reaven, Lithell, & Landsberg, 1996). Indeed, compared to healthy-weight youth, obese children and adolescents have significantly increased LF:HF ratio and decreased HRV parameters indicative of diminished parasympathetic activation (e.g., HF, standard deviation of RR intervals) across ECG recordings of both short- (e.g., 15 min; Chen, Chin, Lee, Sheen, & Jeng, 2012; Nagai & Moritani, 2004; Nagai, Matsumoto, Kita, & Moritani, 2003; Kaufman et al., 2007) and long-duration (24-hours; Riva et al., 2001; Martini et al., 2001). Interestingly, although sympathovagal imbalance among obese youth is observed

during waking hours, it appears to be most robust during the night (Riva et al., 2001; Martini et al., 2001), even after controlling for snoring and sleep apnea (Rabbia et al., 2003). Further, a recent prospective study found that sympathovagal imbalance at age 5.5 years was predictive of obesity five years later, suggesting a causal relation (Graziano, Calkins, Keane, & O'Brien, 2011).

Taken together, research evidence convincingly demonstrates that inadequate sleep is associated with childhood obesity (c.f., Beebe et al., 2006; Chaput et al., 2006; Chaput & Tremblay, 2007; Nielsen, Danielsen, & Sørensen, 2011; Jarrin et al., 2011) and adversely impacts a variety of physiological processes (i.e., autonomic function; Spiegel et al., 1999, 2004). Inadequate sleep, via multiple pathophysiological alterations, thus offers a plausible pathogenic role in the development of obesity. Given that sympathovagal imbalance is related with obesity (c.f., Kaufman et al., 2007; Rabbia et al., 2003; Rodriguez-Colon, Bixler, Vgontzas, & Liao, 2011), it has been proposed as one potential mediator elucidating the association between sleep and the etiology of obesity (c.f., Knutson et al., 2012; Knutson, Spiegel, & Van Cauter, 2008; Spiegel et al., 1999; 2004; Pannain & Van Cauter, 2008).

The aim of the current study was to evaluate whether sympathovagal imbalance was a potential mechanism underlying the association between inadequate sleep and childhood obesity. Inadequate sleep was characterized as short sleep duration, later bed- and rise-times, and sleep disturbances (e.g., sleep-disordered breathing, parasomnia, night wakings). It was posited that sympathovagal imbalance would mediate this relation, even after controlling for important developmentally-relevant covariates.

Method

Study Population

The study population included participants from the QUebec Adipose and Lifestyle InvesTigation in Youth (QUALITY) Cohort. The survey design and methods have been previously reported in detail (Lambert et al., 2011). The aim of the QUALITY study was to investigate the natural history of excess weight and its related cardio-metabolic consequences (e.g., sympathetic hyperactivity) among a large cohort of youth at risk for the development of obesity. Inclusion criteria required at least one biological parent to be overweight (BMI $\geq$30kg/m^2 or waist circumference $\geq$102 cm in men and $\geq$ 88 cm in women). Exclusion criteria included children with serious psychopathology or medical conditions (i.e., diabetes type 1 or 2, hypertension, hospitalized in the last month for a serious illness, renal failure, inflammatory bowel disease, cystic fibrosis), a highly restricted diet (<600 Kcal/day), or medication use of β-blockers or thiazides. All participants were Caucasian of Western European ancestry to reduce genetic admixture.

The baseline data collection (first visit) occurred when youth (N = 630) were aged 8 to 10 years. Participants were invited for the second visit when youth were aged 9 to 12 years. Of the original sample, 89% (n = 564) completed the second visit. Attrition was due to several reasons including refusal of the child (18.2%), family missed several appointments (9%), family had no time, moved away, or could not be located (13.5%), or other reasons not specified (59%). The data used for the present analyses are based on the second visit of data collection when the ECG recordings were collected. The QUALITY study was approved by the ethics review board of Direction Santé Québec,

Institut de la statistique du Québec, and CHU Sainte-Justine Hospital. Informed consent and assent were obtained by parents and youth, respectively.

Measures

Anthropometrics. All anthropometric measures were taken by a registered nurse while participants were dressed in light clothing with shoes off. Using a standard measuring tape, waist circumference was measured at the narrowest part of the body, midway between the lowest rib cage and the iliac crest, and hip circumference was measured at the widest part of the body, over the buttocks. Height was measured during maximal inspiration. Waist and hip circumferences and height were measured in duplicate, to the nearest 0.1 cm; if they differed by more than 0.5 cm, a third measure was taken. The mean of the two closest measures was used in data analyses. Weight was measured to the nearest 0.2 kg with a spring scale tested daily for accuracy and calibrated against a set of standard weights. BMI was calculated as weight in kg divided by height in m^2. Based on age and sex, Z-scores for BMI percentiles were determined using the growth charts published by the U.S. Centers for Disease Control and Prevention (2000). Overweight was defined as BMI in the $\geq 85^{th}$ to $<95^{th}$ age- and sex-specific percentile; obesity was defined as BMI in the $\geq 95^{th}$ percentile.

Dual-energy X-ray absorptiometry (DEXA) scans, considered the gold standard in assessing obesity, were performed using DEXA, Prodigy Bone Densitometer System DFþ14664 (GE Lunar Corporation, Madison, WI, USA). Scan mode was based on weight guidelines provided by the manufacturer and each scan was analyzed using DEXA pediatric software version (Lunar Corporation). Each DEXA scan determined total fat mass (kg/m^2) and percentage of body fat mass [i.e., fat mass/fat mass + lean mass

+ bone mineral content) * 100]. Due to reported differences in the health risks attributable to the distribution of excess adipose tissue (i.e., overall body composition vs. centrally located; Janssen, Katzmarzyk, & Ross, 2004; Janssen, Shields, Craig, & Tremblay, 2011), and in an effort to streamline the results, data reduction techniques were employed to derive composite factors for obesity indices of central adiposity and body composition (see Statistical Analyses).

Sleep. Children reported their typical bed- and rise-time on school and weekend days. Sleep duration for school and weekend days was calculated as the difference between bed- and rise-time. Parents also reported on their child's average bed- and rise-time over the week and average sleep duration was calculated as the difference between bed- and rise-time.

Children's sleep habits were assessed using the Children's Sleep Habits Questionnaire (CSHQ; Owens et al., 2000). Parents answered 33 questions on their child's sleep habits and disturbances over a one week interval. Each question was rated on a 3-point scale that described the frequency (rarely, sometimes, usually) of sleep habits categorized into eight sleep disturbance subscales: sleep duration (e.g., my child sleeps too little), daytime sleepiness (e.g., my child seems tired), sleep-disordered breathing (e.g., my child snorts and gasps), sleep anxiety (e.g., my child is afraid of sleeping alone), sleep onset delay (e.g., my child falls asleep in 20 minutes), night awakenings (e.g., my child awakens more than once), bedtime resistance (e.g., my child struggles at bedtime), and parasomnias (e.g., my child sleepwalks). Items are summed to produce a score for each subscale and a total sleep disturbance score, with higher scores

(>41) reflecting greater sleep disturbances. The CSHQ has demonstrated validity and internal consistency in both community and clinical samples (Owens et al., 2000).

Puberty. Sexual maturation was scored by a pediatric registered nurse according to Tanner descriptions for pre-pubertal, puberty, and post-pubertal stages (Lambert et al., 2011; Marshall & Tanner, 1969, 1970). Criteria used for pre-pubertal stages included no body hair growth, no menstruation or breast growth for girls, and no facial hair growth or deepening of the voice for boys. Criteria for pubertal stage included any indication of body hair and breast growth and or menstruation for girls, and any indication of facial hair growth and or voice changes for boys. Criteria for post-pubertal stages included complete body hair and breast growth, as well as menstruation for girls, and complete facial hair growth and voice changes for boys.

Heart Rate Variability

ECG Data. ECG data were derived from a modified Lead II configuration using disposable, pre-gelled snap silver chloride electrodes. Electrode resistance was minimized (<10 kO) by precleaning the skin with rubbing alcohol swab. The active electrode (and its derivative/dZ) was placed on the right clavicle next to the sternum over the first rib between the two collarbones. The second electrode was placed on the left mid-clavicular line at the apex of the heart over the ninth rib. The ground electrode was placed near the lowest possible right rib cage on the abdomen. Additional dZ electrodes were placed over the right fourth intercostal space at the sternal edge, the fifth intercostal space at the left axillary line, and on the sixth rib in the mid-clavicular line.

ECG Signal Processing. ECG data were uploaded on the MARS® Holter Analysis Workstation (GE Marquette Medical Systems, Milwaukee, Wisconsin, USA),

where data were formatted for viewing, editing, and analysis. Beat-by-beat visual inspection of the shape, trend, and length of each QRS complex was identified based on standard Marquette algorithms for QRS labeling and further verified by visual inspection from a qualified trained professional. The removal of artifacts was based on a 20% change from the previous signal as a criterion (Kleiger, Miller, Bigger, & Moss, 1987). In cases where artifacts and excluded RR intervals were automatically filtered and identified as unreadable signals, the remaining acceptable beats were used to replace the data points via cubic spline interpolation method. At least four acceptable R-peaks were needed in order for spline interpolation to identify the continuous function between two middle R-peaks. Next, input samples were linearly detrended, mean-centered, and tapered using a Hanning window, and processed by FFT periodogram spectrum method. Frequency-domain variables included LF (0.04-0.15 Hz), HF (0.15-0.4 Hz), and LF:HF ratio and were calculated and expressed in absolute units.

Procedure

During the scheduled visit, parents completed questionnaires (e.g., socio-demographics) and reported on child medication use in the last two weeks (e.g., antibiotics, pain/fever, colds/allergies) prior to data collection. Children completed questionnaires on their lifestyle habits (e.g., physical activity, screen time) while seated quietly. Anthropometric measures were collected by a pediatric registered nurse. Next, the nurse prepped for electrode site placement. Raw ECG data were acquired and recorded using the 8500 Marquette MARS Holter monitor (128 Hz; GE Marquette Medical Systems, Milwaukee, Wisconsin, USA). ECG data acquisition began in the morning between 07:00 and 09:00 and lasted ~3 hours. Then, the nurse removed the

electrodes and Holter monitor and after a 5 minute rest, blood pressure was measured. An appropriate-sized occlusion cuff was attached to the medial surface of the right arm over the brachial artery using an oscillometric instrument (model CR9340; Dinamap XL, USA) with demonstrated validity (Park & Menard, 1998) and according to procedures developed by the Child and Adolescent Trial for Cardiovascular Health Program (Webber et al., 1995). While the child was seated, three consecutive measures were obtained at 1 min intervals. The average of the last two measures was used in the present analyses. To reduce violations of stationarity, the procedure was kept consistent for all participants (Berntson et al., 1997). Finally, DEXA scans were conducted to obtain body composition measures.

Statistical Analysis

All data were entered and double-checked by the senior data coordinator and analyzed with IBM SPSS Statistics 20 software (SPSS, Inc., Chicago, IL). Data were retained as continuous to maximize statistical power and were checked for normality and linearity. The LF and HF distributions were skewed and thus, natural log-transformed (ln). For data reduction, factor analysis of the obesity measures (principal component with varimax rotation) yielded two factors: central adiposity (R^2 = 92.8%; Factor loadings: waist circumference = 0.96, hip circumference = 0.96) and body composition (R^2 = 91.9%; Factor loadings: BMI percentile Z-score = 0.94, percent body fat = 0.97, fat mass index = 0.97). For parsimony, only the results with the central adiposity and body composition factors scores are reported in the present study. (All analyses were also tested with each individual obesity measure and results were identical.)

A planned sequence of regression analyses were conducted to test the hypothesis that sympathovagal imbalance would mediate the association between inadequate sleep and childhood obesity (Baron & Kenny, 1986; MacKinnon & Dwyer, 1993; MacKinnon, Warsi, & Dwyer, 1995). First, to test the association between sleep and obesity, the general linear model was used to univariately test each sleep measure predicting each obesity factor (central adiposity, body composition), with and without covariates (age, sex, puberty, screen time, physical activity, household income, parental education).

Second, each sleep measure was univariately tested to predict each HRV parameter (LF, HF, LF:HF ratio), with and without covariates (age, sex, puberty, screen time, physical activity, household income, parental education, heart rate, SBP, DBP). Third, each HRV parameter was univariately tested to predict each obesity factor, with and without covariates. Finally, to initially test for potential mediation, each sleep measure was tested to predict each obesity factor, while controlling for each HRV parameter. Sobel tests were used to calculate the critical ratio as a test of whether the indirect effect of the sleep measure on the obesity index via sympathovagal imbalance was significantly different from zero (MacKinnon, Warsi, & Dwyer, 1995; Sobel, 1982)

Results

Of the 564 participants who completed the second visit of the QUALITY study, 5 were excluded for insufficient data on sleep measures, 67 were excluded because they did not have an ECG recording completed, and 3 were excluded due to ECG recording durations that were less than 30 min, yielding a final sample size of 489. All ECG recordings were reviewed by a board-certified cardiologist; no cardiovascular pathology was identified (i.e., bradycardia, fibrillation, premature contraction). All children were

Caucasian, half were male (56.4%), aged 11.67 years (*SD* = 0.95), of normal weight status (57.7% BMI 5-85th percentile), and most were categorized into pre-pubertal status (64.8%). The majority of children did not take medication two weeks prior to the study for pain, allergies, cold, digestive, cholesterol, or skin problems (91.9%); none were taking medication for diabetes or hypertension. Children's anthropometric and physiological measures are presented in Table 1. Based on self-report, children's bed-time was 20:54 (*SD* = 0:38) on school nights and 22:02 (*SD* = 0:57) on weekend nights; they slept ~9 hours on school nights and 10 hours on weekend nights. Based on parent-report, children retired to bed at 20:51 (*SD* = 1:05), slept an average of 10 hours each night weekly, and exhibited sleep disturbances (e.g., sleep anxiety, bedtime resistance; see Table 2).

Hypothesis Testing: Sleep and Obesity

Regression analyses revealed that shorter sleep duration on school nights (β_{avg} = -0.12, p<.01, R_{avg}^2 = 0.12), later school and weekend bed-times (β_{avg} = 0.15, p<.001, R^2_{avg} = 0.13), greater sleep-disordered breathing (β_{avg} = 0.25, p<.001, R^2_{avg} = 0.18) and more parasomnia symptoms (β_{avg} = 0.12, p<.001, R^2 = 0.13) were associated with greater central adiposity and body composition, in adjusted models (Table 3). No other sleep parameters were associated with obesity indices. (Data for other sleep disturbance subscales are not shown for parsimony.)

Hypothesis Testing: Sleep and Sympathovagal Imbalance

Regression analyses revealed that greater LF:HF ratio was significantly associated with shorter sleep duration (β = -0.12, p =.021, R^2 = 0.09), later bed-times (β = 0.13, p<.01, R^2 = 0.09), and more sleep disturbances (β_{avg} = 0.13, p<.01, R^2_{avg} = 0.10), even

after adjusting for covariates (see Table 4). LF and HF were not significantly associated with sleep duration ($\beta_{avg} = 0.05$, $p_{avg} = 0.314$, $R_{avg}^2 = 0.35$), bed-time ($\beta_{avg} = 0.06$, $p_{avg} = 0.649$, $R_{avg}^2 = 0.34$), or sleep disturbances ($\beta_{avg} = -0.02$, $p_{avg} = 0.345$, $R^2_{avg} = 0.35$), in adjusted models.

Hypothesis Testing: Sympathovagal Imbalance and Obesity

Greater central adiposity was significantly associated with higher LF:HF ratio ($\beta = 0.18$, $p<.001$, $R^2 = 0.25$) and LF ($\beta = 0.12$, $p<.05$, $R^2 = 0.22$), but not with HF ($\beta = -0.03$, $p = 0.603$, $R^2 = 0.22$), in adjusted models (see Table 5). Similarly, greater body composition was significantly associated with higher LF:HF ratio ($\beta = 0.14$, $p<.01$, $R^2 = 0.14$) and LF ($\beta = 0.12$, $p<.05$, $R^2 = 0.12$), but not with HF ($\beta = -0.01$, $p = 0.849$, $R^2 = 0.12$), in adjusted models.

Hypothesis Testing: Mediation

Sympathovagal imbalance was tested as a possible mediator underlying the relation between inadequate sleep and obesity. Regression analyses revealed that sympathovagal imbalance significantly mediated the effect of sleep duration ($R^2 = 0.23$, $\Delta R^2 = 0.03$, $p<.001$; Sobel $z = -2.00$, $p = .045$), bed-time on school nights ($R^2 = 0.25$, $\Delta R^2 = 0.02$, $p<.001$; Sobel $z = 2.05$, $p = .040$), and sleep-disordered breathing symptoms ($R^2 = 0.28$, $\Delta R^2 = 0.02$, $p<.001$; Sobel $z = 2.34$, $p = .019$) on the central adiposity factor. Sympathovagal imbalance also significantly mediated the effect of sleep-disordered breathing on the body composition factor ($R^2 = 0.16$, $\Delta R^2 = 0.01$, $p<.05$; Sobel $z = 2.04$, $p = .041$); and, there was a trend for sleep duration ($R^2 = 0.14$, $\Delta R^2 = 0.01$, $p<.01$; Sobel $z = -1.82$, $p = .069$) and bed-time on school nights ($R^2 = 0.12$, $\Delta R^2 = 0.01$, $p<.01$; Sobel $z =$

1.85, p = .063). (Identical results were obtained when analyses were conducted on the five obesity measure separately; factors are reported for parsimony.)

Discussion

Obesity is a risk factor for multiple chronic diseases, disability, and premature death. Researchers continue to seek a better understanding of the pathogenesis of obesity. While mounting evidences suggests an association between sleep and obesity, the mechanisms underlying their relation are complex and less clear. The aim of the present study was to investigate whether sympathovagal imbalance was one potential pathophysiological mechanism. The present study found support for the meditational role of sympathovagal imbalance in the relation between inadequate sleep and childhood obesity.

The sample was comprised of a large cohort of children at-risk for developing obesity, based on confirmed parental overweight status. This at-risk sample provided a unique opportunity to explore the research question. Compared to national BMI values reported for similarly-aged children participating in the representative National Health and Nutrition Examination Survey 2009-2010 (Ogdon et al., 2012), average BMI values in the present sample were higher for both girls (21.29 *vs*. 18.5) and boys (21.01 *vs*. 18.3). Children in the current study slept an average of 26 min less, went to bed almost 10 min later, woke up 18 min earlier, and exhibited similar types of sleep disturbances compared to values obtained from a representative sample of 11 administrative regions of Québec (Laberge et al., 2001). Finally, compared to previous studies assessing HRV among obese children of similar age ranges, the present sample exhibited higher LF (e.g., 6.70 *vs*. 7.08) and LF:HF ratio (e.g., 1.20 *vs*. 1.85) and similar HF values (e.g., 6.56 vs.

6.55; Latchman, Mathur, Bartel, Axtell, & De Meersman,2011; Rodriguez-Colon et al., 2011). Compared to previous studies assessing HRV among non-obese children (Jarrin, McGrath, Poirier, Séguin, Séguin, Tremblay, & Paradis, submitted; Latchman et al., 2011; Rodriguez-Colon et al., 2011), the present sample had higher LF showed inconsistent results for HF and LF:HF ratio.

Sleep and Obesity

Among this at-risk sample, children with larger central adiposity body and composition had inadequate sleep compared to their lean counterparts. These observed findings are consistent with previous studies (c.f., Knutson & Van Cauter, 2008; Liu et al., 2011; Marshall et al., 2008). Specifically, heavier youth reported shorter sleep duration, later bed- and rise-time, and exhibited more sleep disturbances, (i.e., sleep-disordered breathing, parasomnia), as compared to lean youth, after adjusting for obesity-related covariates. In contrast to previous findings, sleep disturbances, including sleep-onset delay, sleep anxiousness, daytime sleepiness, and bedtime resistance were less consistently associated with obesity (Beebe et al., 2006).

Sympathovagal Imbalance with Sleep and Obesity

Inadequate sleep was associated with sympathovagal imbalance. Curiously, this relation was evident with LF:HF ratio, the most ubiquitous measure of sympathovagal imbalance, but it was not significant for LF or HF measures separately. This may be attributable to the use of ECG recordings obtained during the day; past studies report considerable differences in sympathovagal imbalance during the night (Rabbia et al., 2003; Riva et al., 2001). Consistent with previous studies (c.f., Rodriguez-Colon et al., 2011; Stamatakis & Punjabi, 2010; Walters et al., 2009), children in the present study

with shorter sleep duration, later bed-time on school nights, and more sleep disturbances, showed greater LF:HF ratio, indicative of greater sympathovagal imbalance. This relation remained significant even after controlling for important developmentally-relevant covariates (e.g., heart rate, SBP, DBP; cf., Jarrin et al., submitted). Although LF and HF were not significant, it is not the absolute levels of LF and HF that matter, but rather, their relative contribution to the *imbalance* that is critical (Berntsen et al., 1997, 2007). Finally, similar to previous findings (c.f., Kaufman et al., 2007; Latchman et al., 2011; Rodriguez-Colon et al., 2011), the present study found that children with larger central adiposity and body composition evidenced sympathovagal imbalance, irrespective of numerous covariates.

Sympathovagal Imbalance Mediates Sleep and Obesity

The present results were largely consistent with the hypothesis that greater sympathovagal activity was a pathophysiological mechanism underlying the association between inadequate sleep and obesity in children. Sympathovagal imbalance partially mediated the relation between central adiposity with short sleep duration, later bed-time, and sleep disturbances. Sympathovagal imbalance was identified as a possible mediator underlying the relation between body composition and sleep disturbances. Similarly, a trend was observed for sympathovagal imbalance partially mediating the relation between body composition with short sleep duration and later bed-time on school nights. This potential mediator was selected on apriori theoretical grounds as a representative variable shown to be related to sleep (Speigal et al., 2004) and obesity indices (Rabbia et al., 2003). It was used as initial test of mediation, however, to more convincingly

demonstrate mediation, temporal data (e.g., longitudinal or experimental) is needed to verify causality among variables (Kraemer, Stice, Kazdin, Offord, & Kupfer, 2001).

The current study corroborates past research linking sleep disturbances (i.e., sleep-disordered breathing) with obesity and sympathovagal imbalance (Hakim, Gozal, & Kheirandish-Gozal, 2012). Sleep disturbances are associated with nocturnal arousals, and even brief arousals during sleep produce an increase in heart rate and blood pressure (Ekstedt et al., 2004; Sforza, Chapotot, Pigeau, Naitoh, & Buguet, 2004). In fact, both heart rate and blood pressure can remain elevated for up to 40 sec after a micro-arousal (Blasi et al., 2003; Sgoifo et al., 2006). Compared to normal sleepers, individuals with sleep disorders (e.g., sleep apnea, insomnia) evidence elevated heart rate and a progressive increase in sympathetic activity over time during sleep and wakefulness (Narkiewicz & Somers, 2003), as well as reduced parasympathetic activity (Bonnet & Arand, 2010).

Given that HRV is influenced by circadian rhythm, circadian misalignment (i.e., phase shift or sleeping at inappropriate times) may disrupt the balance of the autonomic nervous system (Massin et al., 2000). In particular, the timing of sleep may disrupt the circadian function of the autonomic nervous system (Haqq et al., 2012; Jarrin, McGrath, & Drake, submitted). Later bed-times are associated with reduced HRV (Jarrin et al., submitted) and greater BMI in children and adolescents (Olds, Maher, & Matricciani, 2011). Adolescents with later bedtimes are more susceptible to overeating and consume more fast-food and caffeinated drinks, compared to those with earlier bedtimes (Fleig & Randler, 2009; Olds, Blunden, Dollman, & Maher, 2010; Snell et al., 2007). Also, adults with later bed-times show an increased reactivity to stress, heart rate, blood pressure, and

sympathovagal imbalance under normal and stressful conditions, compared to individuals with earlier wake-times (Roeser, et al., 2012). Given the salient role of sympathovagal imbalance on multiple dimensions of sleep and obesity, it is quite plausibly a pathogenic mechanism.

Psychophysiology of Sympathovagal Imbalance

Given that most endocrine organs are sensitive to changes in sympathovagal imbalance (Knutson & Van Cauter, 2008), this may lead to pathophysiological consequences that promote obesity, including changes in appetitive hormones (decreased leptin, increased ghrelin), insulin sensitivity, and cortisol (c.f., Bodosi, Gardi, Hajdu et al., 2004; Knutson 2012; Mullinton, Chan, Van Dongen et al., 2003; Taheri, Lin, Austin, Young, & Mignot, 2004; Van Cauter, 2008). In fact, an interaction between sympathetic activation and hormone production exists. For instance, pharmacologic sympathetic blockade increases leptin levels, and after acute treatment with catecholamines, decreases circulating leptin (Rayner & Trayhurn, 2001). Further lines of research indicate that in addition to hormones, white adipose tissue is directly controlled by the parasympathetic and sympathetic branches (Bartness & Bamshad, 1998; Fliers et al., 2003; Youngstrom & Bartness, 1998).

While high parasympathetic input increases adipose mass, sympathetic input decreases fat mass via reduced differentiation and cell proliferation (Fliers et al., 2003; Kreir et al., 2002). Although this seems counterintuitive, white adipose tissue releases significant cytokines and hormones (e.g., leptin, adiponectin, resistin, tumour necrosis factor-alpha) that regulate energy expenditure, appetite, and satiety (Farooqui & O'Rahilly, 2009; Fliers et al., 2003). In other words, hormones that curb the

development of obesity are not produced, as an indirect result of sympathetic overactivation. Research also suggests that the regulation of adipose tissue distribution (i.e., visceral *vs*. subcutaneous) is controlled by selective branches within the autonomic nervous system (Fliers et al., 2003). Among human males, abdominal visceral fat is associated with sympathetic activation, while subcutaneous fat is not (Alvarez, Beske, Ballard & Davy, 2002; Alvarez, Ballard, Beske, & Davy, 2004).

The present findings provide new knowledge about the role of a potential pathogenic pathway (i.e., sympathovagal imbalance) in which inadequate sleep leads to obesity in children. It is postulated that inadequate sleep is a major stressor that activates the stress response system, and if chronic, may cause progressive wear and tear, or allostatic overload (McEwen, 2002, 2006). Chronic stress activation (e.g., frequent sleep disturbances) can lead to negative alterations in the nervous (e.g., sympathovagal imbalance), endocrine (e.g., cortisol; Rueegenberg, Wrosch, & Miller, 2012; Wrosch, Lupien, Miller, & Pruessner, 2008), and immune systems (Gouin, Glaser, Malarkey, Beversdorf, & Kiecolt-Glaser, 2012). Further, physiological dysregulations have been cross-sectionally and longitudinally observed in youth (Low, Salomon, & Matthews, 2009; Matthews, Salomon, Kenyon, & Allen, 2002) and suggested to exert enduring effects on ageing and health (Danese & McEwen, 2012).

While individuals often do not report feeling stressed under conditions of sleep loss, physiological evidence suggests otherwise (Speigel et al., 1999, 2004; Knutson & Van Cauter, 2008). Indeed, following nights of inadequate sleep (i.e., short sleep duration, sleep disturbances), enhanced sympathetic activity has been documented, leading to increases in urinary catecholamine levels, heart rate, blood pressure, and

sympathovagal imbalance among adults and youth (c.f., Lusardi et al., 1996; Rodriguez-Colon et al., 2011; Speigel et al., 1999, 2004; Tochikubo et al., 1996; Walter et al., 2009). Thus, if chronic, allostatic overload may accelerate and exaggerate pathophysiology (McEwen, 2002).

Limitations, Strengths, and Future Recommendations

One limitation was the use of subjective measures to obtain sleep duration, bed- and rise-time, and sleep disturbances. Objective measures of sleep are advantageous, as they can precisely capture sleep dimensions that cannot be assessed with subjective reports, such as sleep efficiency (i.e., time spent in bed actually sleeping), micro-arousals, sleep architecture (i.e., time spent in each sleep stage), and diagnosed sleep disorders (e.g., periodic leg movements, sleep apnea). However, self- and parent-report sleep measures have established reliability and validity within pediatric samples (Owens et al., 2000; Wolfson & Carskadon, 1998; Wolfson et al., 2003). Further, subjective measures of sleep may yield unique information that cannot be objectively measured (e.g., perception of sleep restoration; Van Egeren, Haynes, Franzen, & Hamilton, 1983; Weaver, Kapur, & Yueh, 2004).

A second limitation was that ECG recordings were obtained during the day. Previous studies observed a more prominent nocturnal decrease in parasympathetic activity and sympathetic hyperactivity in obese children (Rabbia et al., 2003; Riva et al., 2001). Interestingly, the relative shift in autonomic modulation (LF:HF ratio), rather than absolute values of LF and HF, may be a more sensitive measure of cardiac autonomic dysfunction. The present findings are largely consistent with experimental studies that demonstrated total and partial sleep deprivation led to substantial increases in

sympathovagal imbalance the following day and persisted up to two days later (Sgoifo et al., 2006; Spiegel et al., 1999, 2004; Tochibuko et al., 1996; Zhong et al., 2005). In fact, adequate amounts of sleep duration have been found to buffer long-term elevations of increased cortisol secretion among older adults over four years (Rueggeberg et al., 2012). Sleep provides respite and recovery time for the heart and body. It is posited that insufficient recovery time via chronic exposure to inadequate sleep may exacerbate autonomic dysfunction. Future studies should investigate the meditational role of HRV in the relation between sleep and obesity, using ECG recorded during the night.

One strength of the study was the large sample comprised of children at-risk for obesity, which provided an exceptional opportunity to investigate the meditational role of sympathovagal imbalance in a targeted, vulnerable sample. In fact, the present sample had higher rates of obesity, poorer sleep, and diminished HRV compared to normative data of similar-aged youth. Second, data collection and the methodological procedure was standardized across participants. Third, the present study included objective measures of obesity and HRV, with equipment recognized as the gold-standard in medical fields (DEXA scan; 8500 Marquette MARS Holter monitor). Finally, the present findings were robust, even when controlling for multiple covariates including puberty, heart rate, blood pressure, and socioeconomic status.

Future studies should incorporate both subjective and objective measures for sleep (e.g., polysomnography) while measuring HRV over longer periods of time (e.g., 48 hours), to more convincingly demonstrate a temporal relation between sleep, sympathovagal imbalance, and child obesity. Sympathovagal imbalance during sleep should also be further examined. For example, spectral coherence analysis between HRV

and cortical electrophysiological signals (e.g., beta, gamma, delta) may more comprehensively elucidate the relation between parasympathetic modulation and cortical activity across distinct sleep stages. In fact, coherence analysis has already been used to examine how HRV and sleep are affected by different sleep disorders (e.g., insomnia, obstructive sleep apnea; Jurysta et al., 2006, 2009). Cardiopulmonary coupling could also be used in conjunction with HRV analyses to disentangle non-stationarity (i.e., noise) and pure signal recorded during sleep (Thomas et al., 2007).

Conclusion

The present findings suggest sympathovagal imbalance may play an important mediational role underlying the association between short sleep duration, later bed-times, and sleep disturbances with childhood obesity. These findings highlight the importance of better understanding sympathovagal imbalance and its plausible role in the etiology and maintenance of obesity. Future research should include longitudinal designs to test the temporal order of this association and comprehensive assessment of sympathovagal imbalance during sleep.

Table 1
Anthropometric and Physiological Measures

Variable	*Total*		*Girls* (n=213)		*Boys* (n=276)	
	Mean (n)	*SD (%)*	*Mean (n)*	*SD (%)*	*Mean (n)*	*SD (%)*
Age (years)	11.66	0.95	11.61	0.97	11.70	0.93
Anthropometrics						
Waist circumference (cm)	71.85	11.95	71.52	11.84	72.0	12.06
Hip circumference (cm)	85.71	10.98	86.81	10.87	84.87	11.01
Body mass index (%^{percentile})	68.69	28.76	68.96	28.22	68.48	29.23
Body mass index (kg/m^2)	21.14	4.85	21.29	4.83	21.01	4.87
Percent body fat (%)	28.54	10.93	31.37	10.04	26.35	11.10
Fat mass index (kg)	6.26	3.58	6.85	3.53	5.80	3.56
Body weight status[1]						
Normal (5th - <85$^{th\ percentile}$)	(282)	(57.7%)	(121)	(56.8%)	(161)	(58.3%)
Overweight (85th - 95$^{th\ percentile}$)	(74)	(15.1%)	(36)	(16.9%)	(38)	(13.8%)
Obesity ($\geq$95$^{th\ percentile}$)	(123)	(25.2%)	(50)	(23.5%)	(73)	(26.4%)
Underweight (<5$^{th\ percentile}$)	(10)	(2%)	(6)	(2.8%)	(4)	(1.4%)
Physiological Data						
Systolic blood pressure (mmHg)	99.91	7.88	98.64	7.56	100.89	7.99
Diastolic blood pressure (mmHg)	49.81	4.73	49.93	4.74	49.71	4.74
Heart rate (beats/min)	77.86	10.17	79.07	9.80	76.93	10.37
LF (ms^2)	1464.51	1081.64	1346.79	1028.35	1555.37	1114.38
ln LF	7.08	0.63	7.01	0.59	7.13	0.66
HF (ms^2)	922.72	681.37	881.36	629.41	954.65	718.40
ln HF	6.55	0.78	6.52	0.76	6.57	0.80
LF:HF ratio	1.85	0.95	1.75	0.73	1.92	1.09

Note. LF = Low frequency (0.04-0.15 Hz); ln = log-transformed value; HF = High frequency (0.1500-0.4 Hz); ms = milliseconds.
[1]Distribution of age-and sex-specific body weight based on Centers for Disease Control values

Table 2

Demographic Measures of Sleep Dimensions in Youth

	Total		*Girls* (n=213)		*Boys* (n=276)	
Variables	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Sleep Duration (min)						
School nights	588.53	40.28	587.61	41.30	589.23	39.52
Weekend nights	612.21	66.53	624.68	66.10	602.51	65.34
Average	594.51	40.90	595.44	42.60	593.79	39.62
Sleep Patterns (hr:min)						
School bed-time	20:54	0:38	20:56	0:39	20:52	0:38
School rise-time	06:41	0:31	06:43	0:31	06:40	0:31
Weekend bed-time	22:02	0:57	22:09	0:58	21:57	0:56
Weekend rise-time	08:13	1:16	08:32	1:09	07:59	1:19
Average bed-time	20:51	1:05	20:50	1:32	20:52	0:33
Average rise-time	06:48	0:38	06:52	0:43	06:46	0:34
Sleep Disturbances[c]						
Sleep behavior problems (range 17-58)	37.81	5.53	38.02	5.10	37.65	5.83
Daytime sleepiness (range 0-20)	9.69	2.96	9.95	3.00	9.50	2.92
Bedtime resistance (range 1-16)	6.48	1.24	6.55	1.29	6.43	1.20
Sleep onset delay (range 1-3)	1.41	0.69	1.39	0.68	1.41	0.69
Sleep duration (range 1-9)	3.51	1.09	3.48	1.06	3.54	1.12
Sleep anxiety (range 1-12)	4.50	1.06	4.52	1.03	4.49	1.08
Night awakenings (range 1-7)	3.27	0.69	3.23	0.62	3.29	0.73
Parasomnia (range 1-15)	7.93	1.54	7.85	1.45	8.00	1.61
Sleep disturbed breathing (range 1-8)	3.25	0.71	3.23	0.63	3.27	0.77

Note. hr : min = hour : minutes. [a]Child-report. [b]Parent-report.[c]Children's Sleep Habits Questionnaire.

Table 3

Beta Regression Coefficients for Sleep predicting Obesity in Adjusted Models

	Adjusted Models							
	Central Adiposity				Body Composition			
	β	*t*	*p*	R^2	*β*	*t*	*p*	R^2
Sleep Duration (min)								
School nights[a]	**-0.12**	**-2.49**	**0.01**	**0.18**	**-0.13**	**-2.46**	**0.01**	**0.07**
Weekend nights[a]	-0.07	-1.50	0.13	0.16	-0.07	-1.54	0.12	0.06
Average[b]	-0.08	-1.85	0.06	0.17	-0.08	-1.71	0.08	0.07
Sleep Patterns (hr:min)								
School bed-time[a]	**0.15**	**2.86**	**0.00**	**0.17**	**0.16**	**3.01**	**0.00**	**0.08**
School rise-time[a]	0.00	0.06	0.95	0.16	0.01	0.30	0.76	0.05
Weekend bed-time[a]	**0.13**	**2.69**	**0.00**	**0.17**	**0.15**	**3.11**	**0.00**	**0.08**
Weekend rise-time[a]	0.01	0.38	0.69	0.16	0.03	0.66	0.50	0.07
Average bed-time[b]	**0.08**	**1.96**	**0.05**	**0.17**	0.07	1.51	0.13	0.07
Average rise-time[b]	-0.02	-0.54	0.58	0.16	-0.00	-0.12	0.90	0.07
Sleep Disturbances[c]								
Sleep-disordered breathing[b]	**0.25**	**5.83**	**0.00**	**0.27**	**0.24**	**2.87**	**0.00**	**0.12**
Parsomnia[b]	**0.13**	**2.93**	**0.01**	**0.18**	**0.11**	**2.43**	**0.01**	**0.08**

Note. *β* = standardized coefficient. *t* = computed value of t-test. hr : min = hour : minutes. Adjusted models include all covariates (age, sex, puberty, screen time, physical activity, household income, parental education).

[a]Child-report. [b]Parent-report. [c]Children's Sleep Habits Questionnaire.

Table 4

Beta Regression Coefficients for Sleep predicting Sympathovagal Imbalance in Adjusted Models

	Adjusted Models											
	LF				HF				LF:HF ratio			
	β	*t*	*p*	R^2	*β*	*t*	*p*	R^2	*β*	*t*	*p*	R^2
Sleep Duration (min)												
School nights[a]	0.03	0.61	0.54	0.36	0.07	1.72	0.08	0.34	**-0.12**	**-2.13**	**0.02**	**0.10**
Weekend nights[a]	**0.09**	**2.18**	**0.02**	**0.36**	0.05	1.37	0.16	0.34	0.03	0.65	0.51	0.08
Average[b]	-0.02	-0.48	0.63	0.36	0.00	0.04	0.96	0.34	-0.05	-0.97	0.33	0.09
Sleep Patterns (hr:min)												
School bed-time[a]	0.06	1.38	0.16	0.36	-0.01	-0.23	0.81	0.33	**0.13**	**2.36**	**0.02**	**0.10**
School rise-time[a]	**0.08**	**2.16**	**0.03**	**0.36**	0.07	1.83	0.07	0.34	-0.01	-0.36	0.71	0.09
Weekend bed-time[a]	-0.01	-0.26	0.68	0.36	-0.03	-0.65	0.92	0.33	0.02	0.47	0.63	0.09
Weekend rise-time[a]	**0.09**	**2.35**	**0.01**	**0.36**	0.05	1.38	0.16	0.34	0.04	0.93	0.35	0.09
Average bed-time[b]	0.02	0.67	0.49	0.36	0.01	0.41	0.68	0.33	0.01	0.22	0.82	0.08
Average rise-time[b]	-0.02	-0.75	0.45	0.36	-0.03	-0.90	0.36	0.34	0.01	0.39	0.69	0.09
Sleep Disturbances[c]												
Sleep-disordered breathing[b]	0.04	1.10	0.22	0.36	-0.03	-0.81	0.53	0.33	**0.13**	**2.87**	**0.00**	**0.10**
Parsomnia[b]	-0.02	-0.44	0.94	0.36	0.04	1.02	0.62	0.34	0.04	1.03	0.28	0.09

Note. *β* = standardized coefficient. *t* = computed value of t-test. hr : min = hour : minutes; LF = Low frequency (0.04-0.15 Hz); ln = log-transformed value; HF = High frequency (0.1500-0.4 Hz); ms = milliseconds. Adjusted models include all covariates (age, sex, puberty, screen time, physical activity, household income, parental education).

[a]Child-report. [b]Parent-report. [c]Children's Sleep Habits Questionnaire.

Table 5

Beta Regression Coefficients for Sympathovagal Balance predicting Obesity in Adjusted Models

	Adjusted Models							
	Central Adiposity				Body Composition			
	β	*t*	*p*	R^2	*β*	*t*	*p*	R^2
Sympathovagal Balance								
ln LF	**0.12**	**2.32**	**0.02**	**0.22**	**0.12**	**2.29**	**0.02**	**0.12**
ln HF	-0.03	-0.50	0.60	0.22	-0.01	0.19	0.84	0.12
LF:HF ratio	**0.18**	**4.12**	**0.00**	**0.25**	**0.14**	**2.92**	**0.04**	**0.14**

Note. *β* = standardized coefficient. *t* = computed value of t-test. hr : min = hour : minutes; LF = Low frequency (0.04-0.15 Hz); ln = log-transformed value; HF = High frequency (0.1500-0.4 Hz); ms = milliseconds. Adjusted models include all covariates (age, sex, puberty, screen time, physical activity, household income, parental education, heart rate, systolic and diastolic blood pressure).

TRANSITION TO GENERAL DISCUSSION

The aim of manuscript 4 was to investigate whether sympathovagal imbalance was one potential pathophysiological mechanism underlying the link between sleep and obesity. The present study found plausible support for the meditational role of sympathovagal imbalance in the association between inadequate sleep and obesity in children. It is recognized that to more conclusively test for mediation, temporal or longitudinal data are required to examine whether changes in sleep cause changes in sympathovagal balance (Kraemer et al., 2001).

In comparison to the normative HRV values derived in obese pediatric populations, children who were at-risk for obesity had higher LF and LF:HF ratio and similar HF (Latchman, Mathur, Bartel, Axtell, & De Meersman, 2011; Rodriguez-Colon et al., 2011). Compared to non-obese children, LF was higher and HF and LF:HF ratio showed inconsistent results compared to other studies (Jarrin, McGrath, Poirier, Séguin, Séguin, Tremblay, & Paradis, submitted; Latchman et al., 2011; Rodriguez-Colon et al., 2011). As hypothesized, sympathovagal imbalance, as represented by LF:HF ratio was significant in the mediation models. Based on previous literature (Kwok et al., 2011; Rodriguez-Colon et al., 2011) and findings from manuscript 2, LF and HF were posited to be associated with inadequate sleep, however, this hypothesis was not supported. This discrepant finding may be attributable to the time of ECG recordings (i.e., day vs. night; Rabbia et al., 2003; Riva et al., 2001) or the at-risk sample used in the present study. Robust differences in parasympathetic modulation are reported in sleep studies assessing nocturnal HRV (Chung et al., 2009; Neilson et al., 2010). It is plausible that daytime HRV may not be as salient an indicator of sympathovagal imbalance as night HRV,

especially as it relates to sleep. Future research should consider testing this association with HRV acquired while sleeping. Altogether, manuscript 4 was an original contribution suggesting that sympathovagal imbalance may be a potential pathophysiological mechanism underlying the association between sleep and childhood obesity.

GENERAL DISCUSSION

HRV is a valuable quantitative marker of the flexibility and balance of the autonomic nervous system and is used extensively in the literature. The inverse relation between sleep and obesity is robust. Cardiac autonomic dysfunction, as measured by HRV, is a putative mechanism underlying this relation. Upon careful review of the HRV literature, considerable knowledge gaps were identified, particularly within pediatric populations. These gaps included a lack of systematic comparisons across software programs used to derive HRV indices, a lack of normative HRV reference values in children, and a lack of standard covariates when assessing HRV in children. Further, autonomic dysfunction, assessed by HRV, remains to be tested as a possible mechanism linking sleep and childhood obesity.

The overarching goal of my research program was to investigate one potential pathogenic pathway in which inadequate sleep contributes to the etiology of obesity in children. The pathogenesis of obesity is complex and multiple putative pathways have been proposed (Knutson, 2012; Van Cauter, 2007). I was particularly interested in autonomic dysfunction, as indexed by sympathovagal imbalance, given preliminary research findings with adults (Hanlon, & Van Cauter, 2011; Knutson & Van Cauter, 2008; Van Cauter et al., 2007). My dissertation was comprised of four manuscripts.

Manuscript 1 was a timely comparison of the measurement fidelity across contemporary computer software programs used to derive common HRV parameters. The study demonstrated strong to excellent measurement fidelity for all time-domain HRV parameters and for all frequency-domain HRV parameters, except VLF. This study contributes to the field by demonstrating rigorous user-decisions and technical

specifications for nuanced HRV processing details are pivotal in ensuring measurement fidelity across signal processing software programs. This study has important implications for the comparison and synthesis of HRV data across studies.

Manuscript 2 addressed a current knowledge gap in the field and aimed to stimulate harmonization of methodology and reporting of HRV parameters within pediatric populations. It is the first study to provide normative HRV referent values of time- and frequency-domain variables in a large, population-based sample of 10-year-old children. Additionally, it is the first study to systematically assess important covariates of HRV within a pediatric population and to consider their role collectively. The study results yielded unique covariates, specific to each HRV parameter that are crucial during childhood. Of the developmentally relevant covariates tested, sex, heart rate, blood pressure, pubertal status, sleep, and physical activity accounted for significant variance in the HRV parameters.

Manuscript 3 extends past research that exclusively focused on the relation between sleep duration and childhood obesity, to also consider sleep disturbances and sleep patterns. Heavier youth were found to report poor sleep quality, more sleep disturbances, and exhibit a delayed sleep phase pattern, even after controlling for sleep duration. Given that different sleep parameters are associated with unique underlying physiological mechanisms, these results suggest that sleep measures beyond duration may more precisely capture the influences that drive the negative association between sleep and obesity, and thus yield more robust associations.

Manuscript 4 is an original study seeking to investigate whether sympathovagal imbalance was a potential mechanism explaining the relation between multiple sleep

parameters and obesity in children. This study found supporting evidence for sympathovagal imbalance as a possible mediator between short sleep duration, sleep patterns, and sleep disturbances with childhood obesity.

Overall, the findings from each of my dissertation manuscripts represent an original contribution to the fields of psychophysiology, sleep, and obesity. From a psychometric perspective, the measurement fidelity findings highlighted the importance of increasing measurement precision and proper interpretation of HRV. It is critical for researchers to have a strong theoretical understanding of HRV when selecting appropriate technical specifications for signal processing and data cleaning. This manuscript underscores critical issues associated with signal processing that have a profound influence on the accuracy of HRV measurement.

Relatedly, the establishment of normative HRV reference values was an important contribution both experimentally and clinically. It will be important for future researchers to extend this work to broader ages of childhood and adolescence. In addition, this study yielded valuable information regarding developmentally relevant and technical covariates for HRV in children. There is often a misconception that the covariates established within the adult literature can be applied to the pediatric literature, yet critical developmental changes, particularly pubertal status, challenge this assumption. These findings have potential implications to advance the psychophysiology field, as referent values and standard covariates may facilitate comparison and synthesis of previously reported HRV data among pediatric studies.

The association between short sleep duration and obesity is robust, as it has been demonstrated using cross-sectional, longitudinal (prospective and retrospective), and

experimental studies (c.f., Cappuccio et al., 2008; Knutson, 2012; Marshall et al., 2008; Nielsen, 2011). Interestingly, in a review of separate background literatures, sympathovagal imbalance was the common factor implicated in both the obesity literature and the sleep literature. Within the obesity research literature, HRV has been used experimentally to characterize the cardiovascular physiological effects of excess adipose (c.f., Kaufman et al., 2007; Rabbia et al., 2003; Riva et al., 2001). Compared to healthy-weight children, overweight and obese youth consistently show greater sympathovagal imbalance (c.f., Kaufman et al., 2007; Rabbia et al., 2003; Riva et al., 2001). Within the sleep literature, experimental studies demonstrate the direct consequences of sleep loss on significant reductions in HF HRV values and increased LF:HF ratio values (Tochibubo et al., 1996; Zhong et al., 2005). The convergence of these findings led me to the next questions regarding the potential role of sympathovagal imbalance in the relation between sleep and obesity.

Experimental findings with adults have led other researchers to propose sympathovagal balance as a potential pathogenic mechanism (Knutson, 2012; Speigel et al., 1999, 2004; Van Cauter, 2008); however, no studies had tested this association to date. Consistent with the hypothesis, sympathovagal imbalance mediated the link between inadequate sleep and obesity. These findings provide new insight into the relation between inadequate sleep and obesity.

In addition to autonomic dysfunction, there are other potential pathogenic pathways that may explain the association of sleep and obesity. Sleep is associated with multiple physiological changes, (i.e., metabolic, endocrine, and cerebral activity) and psychosocial factors (i.e., stress, socio-economic status); however, there is a paucity of

information available on how these physiological changes may play a pathogenic role in the link between sleep and childhood obesity. Experimental studies show that sleep restriction leads to significantly reduced leptin, insulin resistance, and elevated glucose and cortisol concentrations, even after controlling for multiple covariates (Capaldi, Hanwerger, Richardson, & Stroud, 2005; Spiegel, Knutson, Leproult, Tasali, & Van Cauter, 2005). Interestingly, increased basal cortisol secretion is also associated with longer sleep latency, poor perceived sleep quality, more fragmented sleep, and poor sleep patterns in adults (Vgontzas et al., 2003; Wrosch, Miller, Lupien, & Pruessner, 2008) and children (Hatzinger et al., 2007). It will be important for future studies to examine other metabolic and hormonal profiles (e.g., leptin, ghrelin, cortisol) with distinct subjective and objective sleep dimensions (e.g., sleep fragmentation, sleep architecture, sleep efficiency). Much of the work to date has been conducted in lean healthy young men and results cannot be generalized to children and adolescents. Investigating the cardiovascular, metabolic, and endocrine changes across sleep stages in children may further elucidate other physiologic responses that confer risk for the development and maintenance of obesity.

Limitations, Strengths, and Future Research

The present dissertation has two general limitations that merit discussion. The first limitation was the cross-sectional nature of the studies, which precluded the investigation of a causal relation to be assessed between sleep, childhood obesity, and sympathovagal balance. Importantly, future studies with experimental or longitudinal designs are necessary to test the temporal nature of this relation. While experimental sleep restriction studies have been conducted, few exist with children. Interestingly,

summer camps have been previously used in an innovative and ecologically valid design to assess multiple sleep dimensions in a large group of children (Carskadon et al., 2003). Studies experimentally testing how sleep curtailment affects sympathovagal imbalance in children combined with longitudinal, prospective studies are necessary to better elucidate the association between sleep, HRV, and development of obesity.

The second limitation was that the sleep measures were subjective in all studies. Objective sleep measures (e.g., actigraphy, polysomnography) provide detailed information about sleep architecture, sleep fragmentation, and sleep efficiency that cannot be derived from self-report. Amounts of time spent in different sleep stages has been differentially associated to body composition and central adiposity (Rao et al., 2009; Theorell-Haglow et al., 2010). This is especially important given the evidence of sleep-stage dependence of autonomic functioning (i.e., greater parasympathetic dominance during deep sleep and less parasympathetic dominance during rapid eye movement sleep; Trinder et al., 2001). However, objective sleep measures fail to capture one's perception of how restorative sleep is for them. In fact, some evidence suggests subjective measures are better proxies of sleep complaints than objective sleep measures (Van Egeren, Haynes, Franzen, & Hamilton, 1983; Weaver, Kapur, & Yueh, 2004). In my dissertation, the subjectively reported sleep values were within the ranges reported in previous studies with children of the same age range, ethnicity, and sociodemographic backgrounds (e.g., Laberge et al., 2011). Future studies should include both objective and subjective measures of sleep.

Further, given that sympathovagal imbalance partially mediated the association between sleep and obesity, it will be important to consider other indicators of

sympathovagal imbalance. For example, blood pressure typically drops by 10% to 20% during sleep, which is referred to as "dipping" (Pickering, 1990). Interestingly, individuals who do not show dipping (non-dippers) have poor sleep quality and sleep efficiency (Loredo, Nelesen, Ancoli-Israel, & Dimsdale, 2004) and are at greater risk for hypertension, left-ventricular hypertrophy, and mortality, independent of 24-hour blood pressure and obstructive sleep apnea (Ohkubo et al., 2002; Verdecchia et al., 1990). This suggests another potential autonomic dysfunction pathway that should be further investigated. Although there are studies suggesting a reduced sympathetic response during sleep, there is little information on whether HRV values also "dip" during sleep among a pediatric sample. Cardiopulmonary coupling could also be used in conjunction with HRV analyses to disentangle non-stationarity (i.e., noise) and pure signal recorded during sleep (Thomas et al., 2007). This is an interesting avenue for future study.

Conclusion

Overall, the findings from my dissertation provide new knowledge about distinct sleep dimensions that may inform the underlying pathophysiological mechanisms linking sleep and obesity. Sympathovagal imbalance was identified as one specific mechanism posited to mediate sleep and childhood obesity. This proposed mechanism is congruent with the allostatic load theory postulating that the chronic stress associated with inadequate sleep heightens the physiological consequences that eventually lead to harmful health conditions (Danese & McEwen, 2012; McEwen, 2002). Thus, when sleep is disturbed or reduced, modifications within the cardiovascular system may occur and an allostatic overload develops (e.g., reduction in parasympathetic activity), and if chronic,

may lead to harmful pathological consequences (i.e., obesity; Danese & McEwen, 2012; McEwen, 2002).

REFERENCES

Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Barger, A. C., & Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: A quantitative probe of beat-to-beat cardiovascular control. *Science, 213*, 220 –222.

Allison, D. B., Fontaine, K. R., Manson, J. E., Stevens, J., & Vanltallie, T. B. (1999). Annual deaths attributable to obesity in the United States. *Journal of the American Medical Association, 282,* 1530-1538.

Al Mamun, A., Lawlor, D. A., Cramb, S., O'Callaghan, M., Williams, G., & Najman, J. (2002). Do childhood sleeping problems predict obesity in young adulthood? Evidence from a prospective birth cohort study. *Am. J. Epidemiology, 166*, 1368-1373.

Alvarez, G. E., Beske, S. D., Ballard, T. P., & Davy, K. P. (2002). Sympathetic neural activation in visceral obesity. *Circulation, 106*, 2533–2536.

Alvarez, G. E., Ballard, T. P., Beske, S. D., & Davy, K. P. (2004). Subcutaneous obesity is not associated with sympathetic neural activation. *Am J Physiol Heart Circ Physiol., 287*, H414 –H418.

American Sleep Disorders Association. (1990). *International classification of sleep disorders: Diagnostic and coding manual*. Rochester: American Sleep Disorders Association.

Archbold, K. H., Pituch, K. J., Panabi, P., & Chervin, R. D. (2002). Symptoms of sleep disturbances among children at two general pediatric clinics. *The Journal of Pediatrics, 140*, 97–102.

Bailey, J. J., Berson, A. S., Garson, A. Jr., Horan, L. G., Macfarlane, P. W., Mortara, D. W., & Zywietz, C. (1990). Recommendations for standardization and specifications in automated electrocardiography: Bandwidth and digital signal processing: A report for health professionals by an ad hoc writing group of the Committee on Electrocardiography and Cardiac Electrophysiology of the Council on Clinical Cardiology American Heart Association. *Circulation, 81*, 730-739.

Bailey, J. J., Horton, M., & Itscoitz, S. B. (1974). A method for evaluating computer programs for electrocardiographic interpretation. III. Reproducibility testing and the sources of program errors. *Circulation, 50*, 88-93.

Baron, R. M., & Kenny, D. A. (1986). The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *Journal of Personality and Social Psychology, 51,* 1173–1182. doi: 10.1037/0022-3514.51.6.1173

Bartness, T. J., & Bamshad, M. (1998). Innervation of mammalian white adipose tissue: Implications for the regulation of total body fat. *Am J Physiol.,* 275, R1399–R1411.

Baumann, C. R., & Bassetti, C. L. (2005). Hypocretins (orexins) and sleep-wake disorders. *Lancet Neurol, 4*, 673–82.

Bawazeer, N. M., Al-Daghri, N. M., Valsamakis, G., Al-Rubeaan, K. A., Sabico, S. L., Huang, T. T. *et al*. (2009). Sleep duration and quality associated with obesity among Arab children. *Obesity (Silver Spring,) 17*, 2251–2253.

Beebe, D.W., Lewin, D., Zeller, M., McCabe, M., MacLeod, K., Daniels, S.R., & Amin, R. (2006). Sleep in overweight adolescents: Shorter sleep, poorer sleep quality,

sleepiness, and sleep-disordered breathing. *Journal of Pediatric Psychology, 32,* 69-79. doi:10.1093/jpepsy/jsj104

Berntson, G. G., Bigger, J. T., Eckberg, D.L., Grossman, P., Kaufmann, P. G., Malik, M., Nagaraja, H. N., et al. (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology*, *34*, 623–648.

Berntson, G. G., Cacioppo, J. T., & Quigley, K. S. (1991). Autonomic determinism: The modes of autonomic control, the doctrine of autonomic space, and the laws of autonomic constraint. *Psychological Review, 98*, 459–487.

Berntson, G. G., Cacioppo, J. T., & Quigley, K. S. (1993). Cardiac psychophysiology and autonomic space in humans: Empirical perspectives and conceptual implications. *Psychological bulletin*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8416034

Berntson, G. G., Cacioppo, J. T., & Quigley, K. S. (1994). Autonomic cardiac control. I. Estimation and validation from pharmacological blockades. *Psychophysiology*, *31*, 572-585.

Berntson, G. G., Quigley, K. S., Jang, J. F., & Boysen, S. T. (1990). An approach to artifact identification: Application to heart period data. *Psychophysiology*, *27*, 586–598.

Bernston, G. G., Quigley, K. S., & Lozano, D. (2007). Cardiovascular Psychophysiology. In J. T. Cacioppo, L. G. Tassinary, & G. G. Bernston (Eds.), *Handbook of psychophysiology*, 3rd edition, (pp. 182-210). New York: Cambridge University Press.

Berntson, G. G., & Stowell, J. R. (1998). ECG artifacts and heart period variability: Don't miss a beat! *Psychophysiology*, *35*, 127–132.

Białłkowski, J., Karwot, B., Szkutnik, M., Sredniawa, B., Chodor, B., Zeifert, B., Skiba, A., et al. (2003). Comparison of heart rate variability between surgical and interventional closure of atrial septal defect in children. *The American Journal of Cardiology*, *92*(3), 356-358. doi:10.1016/S0002-9149(03)00648-9

Björntorp, P. (2001). Do stress reactions cause abdominal obesity and comorbidities? *Obesity Reviews*, *2,* 73–86.

Bland, J. M., & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, *1*, 307–310.

Bland, J. M., & Altman, D. G. (2003). Applying the right statistics: Analyses of measurement studies. *Ultrasound Obstet Gynecol, 22*, 85–93.

Bloomfield, P. (1976). *Fourier analysis of time series: An introduction.* New York: John Wiley.

Bonner, R. E., Crevasse, L., Ferrer, M. I., & Greenfield, J. C. Jr. (1972). A new computer program for analysis of scalar electrocardiograms. *Comput Biomed Res, 5,* 629–653.

Bonner, R. E., & Schwetman, H. D. (1968). Computer diagnosis of electrocardiograms II: A computer program for EKG measurements. *Comput Biomed Res, 1,* 366–386.

Bonnet, M. H. (2012). Heart rate variability measures add a new dimension to the understanding of sleepiness. *Sleep*, *35*, 307-308.

Bonnet, M. H., & Arand, D. L. (1997). Heart rate variability: Sleep stage, time of night, and arousal influences. *Electroencephalogr Clin Ncurophysiol, 102,* 390-396.

Bonnet, M. H., & Arand, D. L. (2010). Hyperarousal and insomnia: State of the science. *Sleep Medicine Reviews*, *14* (1), 9–15.

Brewster, U. C., Setaro, J. F., & Perazella, M. A. (2003). The renin-angiotensin-aldosterone system: Cardiorenal effects and implications for renal and cardiovascular disease states. *Am J Med Sci., 326(1),* 15-24.

Brotherstone, R., & McLellan, A. (2012). Parasympathetic alteration during sub-clinical seizures. *Seizure, 21*, 391-398. doi:10.1016/j.seizure.2012.03.011

Buchhorn, R., Conzelmann, A., Willaschek, C., Störk, D., Taurines, R., & Renner, T. J. (2012). Heart rate variability and methylphenidate in children with ADHD. *Attention deficit and hyperactivity disorders, 4,* 85-91. doi:10.1007/s12402-012-0072-8

Burton, A. R., Rahman, K., Kadota, Y., Lloyd, A. R., & Vollmer-Conna, U. (2010). Reduced heart rate variability predicts poor sleep quality in a case-control study of chronic fatigue syndrome. *Experimental Brain Research*, *204*, 71-78. doi:10.1007/s00221-010-2296-1

Cacioppo, J. (1994). Social neuroscience: Autonomic, neuroendocrine, and immune responses to stress. *Psychophysiology, 31, 113-128*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8986.1994.tb01032.x/abstract

Cacioppo, J. T., Berntson, G. G., Binkley, P. F., Quigley, K. S., Uchino, B. N., & Fieldston, A. (1994). Autonomic cardiac control. II. Basal response, noninvasice indices, and autonomic space as revealed by autonomic blockades. *Psychophysiology*, *31*, 586-598.

Calamaro, C., Park, S., Mason, T. B., Marcus C. L., Weaver, T. E., Pack, A., et al..

(2010). Shortened sleep duration does not predict obesity in adolescents. *J Sleep Res, 19*, 559-566.

Camm, A. J., Pratt, C. M., Schwartz, P. J., AL-Khalidi, H. R., Spyt, M. J., Holroyde, M. J., Karam, R., et al. (2004). Mortality in patients after a recent myocardial infarction: A randomized, placebo-controlled trial of azimilide using heart rate variability for risk stratification. *Circulation*, *109*, 990–996. doi:10.1161/01.CIR.0000117090.01718.2A

Capa, R. L., Cleeremans, A., Bustin, G. M., & Hansenne, M. (2011). Long-lasting effect of subliminal processes on cardiovascular responses and performance. *Int J Psychophysiol, 81*, 22-30.

Capaldi II, V. F., Handwerger, K., Richardson, E., & Stroud, R. L. (2005). Associations between sleep and cortisol responses to stress in children and adolescents: A pilot study. *Behavioral Sleep Medicine, 3,* 177-192.

Cappuccio, F. P., Taggart, F. M., Kandala, N. B., Currie, A., Peile, E., Stranges, S., & Miller, M. A. (2008). Meta-analysis of short sleep duration and obesity in children and adults. *Sleep, 31,* 619-626.

Carskadon, M. A., Acebo, C., Richardson, G. S., Tate, B.A., & Seifer, R. (1997). Long nights protocol: Access to circadin parameters in adolescents. *J Biol Rhythms*, *12*, 278-289.

Carskadon, M. A., Vieira, C., & Acebo, C. (1993). Association between puberty and delayed phase preference. *Sleep, 16*, 258-262.

Carter, P. J., Taylor, B. J., Williams, S. M., & Taylor, R.W. (2011). Longitudinal analysis of sleep in relation to BMI and body fat in children: The FLAME study.

BMJ, 26, 342-d2712.

Castiglioni, P., Di Rienzo, M., Veicsteinas, A., Parati, G., & Merati, G. (2007). Mechanisms of blood pressure and heart rate variability: An insight from low-level paraplegia. *Am. J. Physiol. Regul. Integr. Comp. Physiol, 292*, R1502–R1509.

Chen, S.-R., Chiu, H.-W., Lee, Y.-J., Sheen, T.-C., & Jeng, C. (2012). Impact of pubertal development and physical activity on heart rate variability in overweight and obese children in Taiwan. *The Journal of school nursing : The official publication of the National Association of School Nurses*. Advance online publication. doi:10.1177/1059840511435248

Chaput, J-P., Brunet, M., & Tremblay, A. (2006). Relationship between short sleeping hours and childhood overweight/obesity: Results from the 'Quebec en Forme' Project. *Int. J Obes., 30,* 1080-1085. doi:10.1038/sj.ijo.0803291

Chaput, J-P., & Tremblay, A. (2007). Does short sleep duration favor abdominal adiposity in children? *Int J Ped. Obes., 2,* 188-191. doi:10.1080/1747716070130614

Chen, X., Beydoun, M. A., & Wang, Y. (2008). Is sleep duration associated with childhood obesity? A systematic review and meta-analysis. *Obesity, 16,* 265-274.

Chen, S.-R., Lee, Y.-J., Chiu, H.-W., & Jeng, C. (2008). Impact of physical activity on heart rate variability in children with type 1 diabetes. *Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery*, *24(6),* 741-747. doi:10.1007/s00381-007-0499-y

Dahl, R. E., & Carskadon, M. A. (1995). Sleep in its disorders in adolescence. In R. Ferber & M. Kryger (Eds.). *Principles and practice of sleep medicine in the child.* Philadelphia: W. B. Saunders.

Danese, A., & McEwen, B. S. (2012). Adverse childhood experiences, allostasis, allostatic load and age-related disease. *Physiology & Behavior*, *106,* 29-39.

Daniels, S. R., Morrison, J. A., Sprencher, D. L., Khoury, P., & Kimball, T. R. (1999). Association of body fat distribution and cardiovascular risk factors in children and adolescents. *Circulation, 99,* 541–545.

Dewald, J. F., Meijer, A. M., Oort, F. J., Kerkhof, G. A., & Bo, S. M. (2010). The influence of sleep quality, sleep duration and sleepiness on school performance in children and adolescents: A meta-analytic review. *Sleep Med Rev, 14,* 179–189.

Diedrich, A., Jordan, J., Tank, J., Shannon, J. R., Robertson, R., Luft, F. C., Robertson, D., & Biaggioni, I. (2003). The sympathetic nervous system in hypertension: Assessment by blood pressure variability and ganglionic blockade. *J Hypertens*, *21,* 1677–1686.

Dietrich, A., Rosmalen, J. G. M., Althaus, M., van Roon, A. M., Mulder, L. J. M., Minderaa, R. B., Oldehinkel, & A. J., Riese, H. (2010). Reproducibility of heart rate variability and baroreflex sensitivity measurements in children. *Biol Psychol*, *85*, 71-78.

Di Lorenzo, L., De Pergola, G., Zocchetti, C., L'Abbate, N., Basso, A., Pannacciulli, N. et al. (2003). Effect of shift work on body mass index: Results of a study performed in 319 glucose-tolerant men working in a Southern Italian industry. *Int J Obs*, *27,* 1353-1358.

Dorn, L. D., Dahl, R. E., Woodward, H. R., & Biro, F. (2006). Defining the boundaries of early adolescence: A user's guide to assessing pubertal status and pubertal timing in research with adolescents. *Appl. Dev. Sci., 10*, 30-56.

Drapeau, V., Therrien, F., Richard, D., & Tremblay, A. (2003). Is visceral obesity a physiological adaptation to stress? *Panminerva Med***,** *45,* 189–95.

Dundaröz, M. R., Denli, M., Uzun, M., Aydin, H. I., Sarici, S. U., Yokuşoğlu, M., & Ulgen, S. (2001). Analysis of heart rate variability in children with primary nocturnal enuresis. *International urology and nephrology*, *32*(3), 393-397. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11771860

Edinger, J. D., Fins, A.I., Glenn, D. M., Sullivan, Jr. R. J., Bastian, L. A., Marsh, G. R., et al. (2000). Insomnia and the eye of the beholder: Are there clinical markers of objective sleep disturbances among adults with and without insomnia complaints? *J Consult Clin Psychol, 68,* 586-593.

Ekstedt, M., Åkerstedt, T., & Söderström, M. (2004). Microarousals during sleep are associated with increased levels of lipids, cortisol, and blood pressure. *Psychosom Med*, 66, 925-931.

Eryonucu, B., Uzun, K., Guler, N., & Bilge, M. (2001). Comparison of the acute effects of salbutamol and terbutaline on heart rate variability in adult asthmatic patients. *Eur Respir J, 17,* 863–867.

Farooqi, I. S., & O'Rahilly, S. (2009). Leptin: A pivotal regulator of human energy homeostasis. *Am J Clin Nutr., 89*, 980S–984S.

Faulkner, M. S., Hathaway, D., & Tolley, B. (2003). Cardiovascular autonomic function in healthy adolescents. *Heart Lung*, *32*, 10-22.

Ferri, R., Curzi-Dascalova, L., Arzimanoglou, A., Bourgeois, M., Beaud, C., Lahorgue, Nunes, M., Elia, M., et al. (2002). Heart rate variability during sleep in children with partial epilepsy. *J Sleep Res*, *11*, 153-160.

Finley, J., & Nugent, S. T. (1995). Heart rate variability in infants, children and young adults. *Journal of the autonomic nervous system*, *51*, 103-108. Retrieved from http://www.sciencedirect.com/science/article/pii/0165183894001173

Finley, J. P., Nugent, S. T., & Hellenbrand, W. (1987). Heart-rate variability in children. Spectral analysis of developmental changes between 5 and 24 years. *Canadian journal of physiology and pharmacology*, *65*(10), 2048-52. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3427543

Fleig, D., & Randler, C. (2009). Association between chronotype and diet in adolescents based on food logs. *Eat Behav., 10,* 115–118.

Fleming, S., Thompson, M., Stevens, R., Heneghan, C., Plüddemann, A., Maconochie, I., Tarassenko, L., et al. (2011). Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: A systematic review of observational studies. *Lancet*, *377*(9770), 1011-1018. doi:10.1016/S0140-6736(10)62226-X

Fliers, E., Kreier, F., Voshol, P. J., Haveke, L.M., Sauerwein, H.P., Kalsbeek, A., Buijs, R.M., & Romijn, J.A. (2003). White adipose tissue: Getting nervous. *J Neuroendocrinol., 15,* 1005–1010.

Franco, P., Seret, N., Van Hees, J. N., Lanquart, J-P., Groswasser, J., & Kahn, A. (2003). Cardiac changes during sleep in sleep-deprived infants. *Sleep, 26,* 845-848.

Freedman, D. S., Dietz, W. H., Srinivasan, S. R., & Berenson, G. S. (1999). The relation of overweight to cardiovascular risk factors among children and adolescents: The

Bogalusa Heart Study. *Pediatrics, 103,* 1175-1182.

Freedman, D. S., Khan, L. K., Serdula, M. K., Dietz, W. H., Srinivasan, S. R., & Berenson, G. S. (2005). The relation of childhood BMI to adult adiposity: The Bogalusa Heart Study. *Pediatrics, 115,* 22-27.

Freedman, D. S., Serdula, M. K., Srinivasan, S. R., & Berenson, G. S. (1999). Relation of circumferences and skinfold thickness to lipid and insulin concentrations in children and adolescents: The Bogalusa Heart Study. *American Journal of Clinical Nutrition, 69,* 308-317.

Friedman, B. H. (2007). An autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone. *Biol Psychol*, *74*, 185–199.

Friedman, B. H., & Thayer, J. F. (1998). Anxiety and autonomic flexibility: A cardiovascular approach. *Biol Psychol, 49*, 303–323.

Fujiwara, J., Kimura, S., Tsukayama, H., Nakahara, S., Haibara, S., Fujita, M., Isobe, N., & Tamura, K. (2004). Evaluation of the autonomic nervous system function in children with primary monosymptomatic nocturnal enuresis-power spectrum analysis of heart rate variability using 24-hour Holter electrocardiograms. *Scand J Urol Nephrol.*, *35*, 350-356.

Furness, J. B. (2006). The organisation of the autonomic nervous system: Peripheral connections. *Autonomic Neuroscience: Basic and Clinical, 130,* 1-5.

Gamelin, F.-X., Baquet, G., Berthoin, S., Thevenet, D., Nourry, C., Nottin, S., & Bosquet, L. (2009). Effect of high intensity intermittent training on heart rate variability in prepubescent children. *European journal of applied physiology*, *105*(5), 731-738. doi:10.1007/s00421-008-0955-8

Gianaros, P. J., & Sheu, L. K., (2009). A review of neuroimaging studies of stressor-evoked blood pressure reactivity: Emerging evidence for a brain-body pathway to coronary heart disease risk. *Neuroimage*, *47,* 922-936.

Gianaros, P. J., Van Der Veen, F. M., & Jennings, J. R. (2004). Regional cerebral blood flow correlates with heart period and high-frequency heart period variability during working-memory tasks: Implications for the cortical and subcortical regulation of cardiac autonomic activity. *Psychophysiology*, *41*, 521–530.

Gerritsen, J., Dekker, J. M., TenVoorde, B. J., Kostense, P. J., Heine, R. J., & Bouter, L.M., et al. (2001). Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: The Hoorn Study. *Diabetes Care, 24,* 1793–1798.

Goldberger, J. J. (1999). Sympathovagal balance: How should we measure it? *American Journal of Physiology, 4,* H1273–H1280.

Golding, Pembrey, Jones and The Alspac Study Team. (2001). ALSPAC–The Avon Longitudinal Study of Parents and Children. *Paediatric and Perinatal Epidemiology, 15*, 74–87. doi: 10.1046/j.1365-3016.2001.00325.x

Goto, M., Nagashima, M., Baba, R., Nagano, Y., Yokota, M., Nishibata, K., & Tsuji, A. (1997). Analysis of heart rate variability demonstrates effects of development on vagal modulation of heart rate in healthy children. *J Pediatr, 130*, 725-729.

Graziano, P.A., Calkins, S. D., & Keane, S. P., & O'Brien, M. (2011). Cardiovascualr regulation profile predicts developmental trajectory of BMI and pediatric obesity. *Obesity (Silver Spring), 19*, 1818-1825.

Gray, M. A., Minati, L., Harrison, N. A., Gianaros, P. J., Napadow, V., & Critchley, H.D. (2009). Physiological recordings: Basic concepts and implementation during functional magnetic resonance imaging. *Neuroiamge, 47*, 1105-1115.

Guo, Y. F., & Stein, P. K. (2003). Circadian rhythm in the cardiovascular system: Chronocardiology. *Am Heart J, 145,* 779–786.

Gouin, J. P., Glaser, R., Malarkey, W. B., Beversdorf, D., & Kiecolt-Glaser, J. K. (2012). Childhood abuse and inflammatory responses to daily stressors. *Annals of Behavioral Medicine* Online advance publication. doi: 10.1007/s12160-012-9386

Hakim, F., Gozal., D., & Kheirandish-Gozal., L. (2012). Sympathetic and catecholaminergic alterations in sleep apnea with particular emphasis on children. *Fron Neurol., 3*, 1-13.

Hale, L., & Berger, L. M. (2011). Sleep duration and childhood obesity: Moving from research to practice. *Sleep*, *34*, 1153-1154.

Hall, M., Vasko, R., Buysse, D., Ombao, H., Chen, Q., Cashmere, J.D., Kupfer, D., & Thayer, J. F. (2004). Acute stress affects heart rate variability during sleep. *Psychosomatic Medicine, 66,* 56-62. doi:10.1097/01.PSY.0000106884.58744.09

Han, L., Ho, T. F., Yip, W. C., & Chan, K. Y. (2000). Heart rate variability of children with mitral valve prolapse. *Journal of Electrocardiology*, *33*(3), 219-224. doi:10.1054/jelc.2000.7661

Hanlon, E. C., & Van Cauter, E. (2011). Quantification of sleep behavior and of its impacton the cross-talk between the brain and peripheral metabolism. *PNAS, 108*, 15609-15616. doi/10.1073/pnas.1101338108

Haqq, A. M., DeLorey, D. S., Sharma, A. M., Freemark, M., Kreier, F., Mackenzie, M. L. & Richer, L. P. (2011). Autonomic nervous system dysfunction in obesity and Prader–Willi syndrome: Current evidence and implications for future obesity therapies. *Clinical Obesity,1,* 175–183. doi: 10.1111/j.1758-8111.2012.00032.x

Harris, F. J. (1978). On the use of windows for harmonic analysis with the discrete fourier transform. *Proc IEEE 66,* 51-83.

Harro, M. (1997). Validation of a questionnaire to assess physical activity of children ages 4–8 years. *Res Q Exerc Sport, 68,* 259–268.

Hatzinger, M., Brand, S., Perren, S., Stadelmann, S., von Wyl, A., von Klitzing, K., & Holsboer-Trachsler, E. (2007). Electroencephalographic sleep profiles and hypothalamic–pituitary–adrenocortical (HPA)-activity in kindergarten children: Early indication of poor sleep quality associated with increased cortisol secretion. *Journal of Psychiatric Research, 42*, 532-543.

Haynes, W. G., Morgan, D. A., Walsh, S. A., Mark, A. L., & Sivitz, W. I. (1997). Receptor- mediated regional sympathetic nerve activation by leptin. *J Clin Invest*., *100,* 270 –278.

He, M., Harris, S., Piche, L., & Beynon, C. (2009). Understanding screen- related sedentary behavior and its contributing factors among school-aged children: A social-ecologic exploration. *Am J Health Promot*, 23, 299–308.

Hemingway, H., Shipley, M., Brunner, E., Britton, A., Malik, M., & Marmot, M. (2005). Does autonomic function link social position to coronary risk? The Whitehall II study. *Circulation 111*, 3071–3077.

Henje Blom, E., Olsson, E. M. G., Serlachius, E., Ericson, M., & Ingvar, M. (2009). Heart rate variability is related to self-reported physical activity in a healthy adolescent population. *European journal of applied physiology*, *106*(6), 877-83. doi:10.1007/s00421-009-1089-3

Heragu, N. P., & Scott, W. A. (1999). Heart rate variability in healthy children and in those with congenital heart disease both before and after operation. *The American journal of cardiology*, *83*(12), 1654-1657. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10392871

Hull, Jr. S. S., Evans, A. R., Vanoli, E., Adamson, P. B., Stramba-Badiale, M., Albert, D. E., Foreman, R. D., & Schwartz, P. J., (1990). Heart rate variability before and after myocardial infarction in conscious dogs at high and low risk of sudden death. *J Am Col Cardiol., 16*, 978-985.

Iglowstein, I., Jenni, O. G., Molinari, L., & Largo, R. H. (2003). Sleep duration from infancy to adolescence: reference values and generational trends. *Pediatrics, 111, 302-307.*

Irwin, M., Thompson, J., Miller, C., Gillin, J. C., & Ziegler, M. (1999). Effects of sleep and sleep deprivation on catecholamine and interleukin-2 levels in human clinical implications. *The Journal of Clinical Endocrinology & Metabolism, 84,* 1979-1985.

Janackova, S., & Sforza, E. (2008). Neurobiology of sleep fragmentation: Cortical and autonomic markers of sleep disorders. *Curr Pharm Des*, 14, 3474-3480.

Janssen, I., Katzmarzyk, P. T., & Ross, R. (2004). Waist circumference and not body mass index explains obesity related health risk. *Am J Clin Nutr., 79,* 379-384.

Janssen, I., Shields, M., Craig, C. L., & Tremblay, M. S. (2011). Prevalence and secular changes in abdominal obesity in Canadian adolescents and adults 1981 to 2007–2009. *Obes. Rev., 12,* 397–405. doi: 10.1111/j.1467-789X.2010.00815.x

Jarrin, D. C., McGrath, J. J., & Drake, C. L. (2011). The Relation between Central Adiposity, Sleep Duration and Sleep Quality in Youth. [Abstract] *Sleep Medicine, 12*, S94.

Jarrin, D. C., McGrath, J. J., & Drake, C. L. (2012, submitted). Beyond sleep duration: Distinct sleep dimensions are associated with obesity in children and adolescents.

Jarrin, D. C., McGrath, J. J., Giovanniello, S., Poirier, P. P., & Lambert, M. (2012). Measurement Fidelity of Heart Rate Variability Signal Processing: The Devil is in the Details. *International Journal of Psychophysiology*.

Jarrin, D. C., McGrath, J. J., Poirier, P. P., Séguin, J. R., Séguin, L., Tremblay, R. E., & Paradis, G. (submitted.) Short-Term Heart Rate Variability in a Population-Based Sample of 10-Year-Old Children**.**

Javaheri, S., Storfer-Isser, A., Rosen, C. L., & Redline, S. (2008). Sleep quality and elevated blood pressure in adolescents. *Circulation, 118*, 1034–1040.

Jennings, J. R., Muldoon, M. F., Hall, M., Buysse, D. J., & Manuck, S. B. (2007). Self-reported sleep quality is associated with the metabolic syndrome. *Sleep, 30,* 219-223.

Jensen-Urstad, K., Storck, N., Bouvier, F., Ericson, M., Lindblad, L. E., & Jensen-Urstad, M. (1997). Heart rate variability in healthy subjects is related to age and gender. *Acta Physiol Scand., 160,* 235-241.

Jetté, M. (2002). Survey description and methodology: Part I. Logistics and longitudinal data collections. In Quebec Longitudinal Study of Child Development (QLSCD 1998–2000, Vol. 2, No. 1). Quebec City, Quebec, Canada: Institut de la Statistique du Quebec. Retrieved December 1, 2005, from http://www.stat.gouv.qc.ca/publications/sante/bebe_v2no1_ an.htm

Jetté, M., Desrosiers, H., & Tremblay, R. E. (1997). '*En 2001 . . . J'aurai 5 ans', enquête auprès des bébés de 5 mois: Rapport préliminaire de l'étude longitudinale du développement des enfants du Québec (ÉLDEQ) [translation: 'In 2001 . . . I'll be 5 years old' survey of 5 months old infants: Preliminary report from the Québec Longitudinal Study of Childhood Development (QLSCD)]*. Montréal, Quebec: Ministère de la Santé et des Services sociaux, Gouvernement du Québec.

Jetté, M., & Des Groseilliers, L. (2000). Survey description and methodology. In Longitudinal study of child development in Quebec (ELDEQ 1998–2000, Vol. 1, No. 1). Quebec City, Quebec, Canada: Institut de la Statistique du Quebec. Retrieved December 1, 2005, from http://www.stat.gouv.qc.ca/publications/sante/bebe_no1_pdf_an.htm

Jezewski, J., Kupka, T., & Horoba, K. (2008). Extraction of fetal heart rate signal as time event series from evenly sampled data acquired using Doppler ultrasound technique. *IEEE Trans Biomed Eng, 55*, 805-810.

Jung, J., Heisel, A., Tscholl, D., Fries, R., Schieffer, H., & Ozbek, C. (1996). Assessment of heart rate variability by using different commercially available systems. *Am J Cardiol, 78*, 118-120.

Jurysta, F., Lanquart, J. P., Sputaels, V., Dumont, M., Migeotte, P. F., Leistedt, S., et al.

(2009). The impact of chronic primary insomnia on the heart rate EEG variability link. *Clin Neurophysiol, 120(6),* 1054e60.

Jurysta, F., Lanquart, J. P., van de Borne, P., Migeotte, P. F., Dumont, M., Degaute, J. P., et al. (2006). The link between cardiac autonomic activity and sleep delta power is altered in men with sleep apnea-hypopnea syndrome. *Am J Physiol Regul Integr Comp Physiol, 291*, R1165e71.

Karacan, M., Ceviz, N., & Olgun, H. (2011). Heart rate variability in children with acute rheumatic fever. *Cardiology Young*, *22*, 1-8. doi:10.1017/S1047951111001429

Kaufman, C. L., Kaiser, D. R., Steinberger, J., & Dengel, D. R. (2007). Relationships between heart rate variability, vascular function, and adiposity in children. *Clinical autonomic research: Official journal of the Clinical Autonomic Research Society*, *17*(3), 165-171. doi:10.1007/s10286-007-0411-6

Kazuma, N., Otsuka, K., Wakamatsu, K., Shirase, E., & Matsuoka, I. (2002). Heart rate variability in normotensive healthy children with aging. *Clinical and experimental hypertension*, *24*, 83-89. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11848172

Kitney, R. (1980). An analysis of the thermoregulatory influences on heart-rate variability. In: Kitney, R.I., Rompelman, O, (Ed.). Oxford, England: Clarendon Press.

Kim, K. K., Kim, J. S., Lim, Y. G., & Park, K. S. (2009). The effect of missing RR-interval data on heart rate variability analysis in the frequency domain. *Physiol Meas, 30,* 1039-1050.

Kim, K. K., Lim, Y. G., Kim, J. S., & Park, K. S. (2007). Effect of missing RR-interval data on heart rate variability analysis in the time domain. *Physiol Meas 28*, 1485-1494.

Kleiger, R. E., Miller, J. P., Bigger, Jr, J. T., & Moss, A. J. (1987). Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *J Am Coll Cardiol, 59,* 256–262.

Kleiger, R. E., Stein, P. K., Bosner, M. S., & Rottman, J. N. (1992). Time domain measurements of heart rate variability. *Cardiology Clinics, 10,* 487-498.

Kligfield, P., Gettes, L., Bailey, J.J., Childers, R., Deal, B.J., Hancock, W., van Herpen, G., et al. (2007). Recommendations for the standardization and interpretation of the electrocardiogram: part I: The electrocardiogram and its technology: A scientific statement from the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society: endorsed by the International Society for Computerized Electrocardiology. *J Am Coll Cardiol, 49*, 1109–1127.

Knutson, K. L. (2005). Sex differences in the association between sleep and body mass index in adolescents. *J Pediatr*, *147*, 830-834.

Knutson, K. L. (2012). Does inadequate sleep play a role in vulnerability to obesity? *Am J Hum Biol. 24*(3):361-71. doi: 10.1002/ajhb.22219.

Knutson, K. L., K. Spiegel, P. Penev & Van Cauter, E. (2007). The metabolic consequences of sleep deprivation. *Sleep Med. Rev., 11,* 163–178.

Knutson, K. L. & van Cauter, E. (2008). Associations between sleep loss and increased

risk of obesity and diabetes. *Ann. N.Y. Acad. Sci., 1129*, 287–304.

Köhler, B. U., Hennig, C., & Orglmeister, R. (2003). The principles of software QRS detection. *IEEE Eng Med Biol*, *21*, 42-57.

Kraemer, H.C., Stice, E., Kazdin, A., Offord, D., & Kupfer, D. (2001). How do risk factors work together? Mediators, moderators, and independent, overlapping, and proxy risk factors. *AmJ Psychiatry, 158,* 848-856.

Kreier, F., Fliers, E., Voshol, P. J., Van Eden, C.G., Havekes, L. M. Van Hejiningen, C. L., et al., (2002). Selective parasympathetic innervation of subcutaneous and intra-abdominal fat-functional implications. *J Clin Invest, 110,* 1243–1250.

Kwok, K.-L., Yung, T.-C., Ng, D. K., Chan, C.-H., Lau, W.-F., & Fu, Y.-M. (2011). Heart rate variability in childhood obstructive sleep apnea. *Pediatric pulmonology*, *210*, 205-210. doi:10.1002/ppul.21268

Laberge, L., Petit, D., Simard, C., Vitaro, F., Tremblay, R. E., & Montplaisir, J. (2001). Development of sleep patterns in early adolescence. *Journal of sleep research*, *10*(1), 59-67. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11285056

Lahey, M. A., Downey, R. G., Saal, F. E. (1983). Intraclass correlations: There's more than meets the eye. *Psychol Bull, 93*, 586-595.

Lahiri, M. K., Kannankeril, P. J., & Goldberger, J. J. (2008). Assessment of autonomic function in cardiovascular disease: Physiological basis and prognostic implications. *J Am Coll Cardiol, 51,* 1725-1733.

Lambert, M., Van Hulst, A., O'Loughlin, J., Tremblay, A., Barnett, T. A., Charron, H., Drapeau, V., et al. (2011). Cohort profile: The Quebec adipose and lifestyle investigation in youth cohort. *Int J Epidemiol,* Epub advance online publication.

doi: 10.1093/ije/dyr111
http://ije.oxfordjournals.org/content/early/2011/07/23/ije.dyr111.short?rss=1

Laposky, A. D., Bass, J., Kohsaka, A., & Turek, F. W. (2008). Sleep and circadian rhythms: Key components in the regulation of energy metabolism. *FEBS Lett,582,* 142–151.

Latchman, P. L., Mathur, M., Bartels, M. N., Axtell, R. S., & De Meersman, R. E. (2011). Impaired autonomic function in normotensive obese children. *Clinical autonomic research: Official journal of the Clinical Autonomic Research Society*, *21*(5), 319-23. doi:10.1007/s10286-011-0116-8

Lauderdale, D. S., Knutson, K. L., Rathouz, P. J., Yan, L. L., Hulley, S. B., & Liu, K. (2009). Cross-sectional and longitudinal associations between objectively measured sleep duration and body mass index. *Am J Epidemiol., 170,* 805–813.

Lenard, Z., Studinger, P., Mersich, B., Kocsis, L., & Kollai, M. (2004). Maturation of cardiovagal autonomic function from childhood to young adult age. *Circulation*, *110*(16), 2307-2312. doi:10.1161/01.CIR.0000145157.07881.A3

Li, Z., Snieder, H., Su, S., Ding, X., Thayer, J.F., Treiber, F.A., & Wang, X. (2009). A longitudinal study in youth of heart rate variability at rest and in response to stress. *Int J Psychophysiol, 73*, 212-217.

Liao, D., Carnethon, M., Evans, G.W., Cascio, W.E., & Heiss, G. (2002). Lower heart rate variability is associated with the development of coronary heart disease in individuals with diabetes: The atherosclerosis risk in communities (ARIC) study. *Diabetes, 51*, 3524–31.

Liao, D., Li, X., Rodriguez-Colon, S. M., Liu, J., Vgontzas, A. N., Calhoun, S., & Bixler, E. O. (2010). Sleep-disordered breathing and cardiac autonomic modulation in children. *Sleep medicine*, *11*(5), 484-848. doi:10.1016/j.sleep.2009.11.012

Lindmark, S., Wiklund, U., Bjerle, P., & Eriksson, J.W. (2003). Does the autonomic nervous system play a role in the development of insulin resistance? A study on heart rate variability in first-degree relatives of Type 2 diabetes patients and control subjects. *Diabet Med, 20*, 399–405.

Lippman, N., Stein, K. M., & Lerman, B. B. (1994). Differential therapeutic responses of patients with isoproterenol-dependent and isoproterenol-independen vasodepressor syncope. *Am Heart J, 128*, 1110-1116.

Liu, J., Hay, J., Joshi, D., Faught, B. E., Wade, T., & Cairney, J. (2011). Sleep difficulties and obesity among preadolescents. *Canadian Journal of Public Health Revue Canadienne De Sante Publique, 102*(2), 139-143.

Liu, X., Uchiyama, M., Okawa, M., & Kurita, H. (2000). Prevalence and correlates of self-reported sleep problems among Chinese adolescents. *Sleep, 23,* 27-34.

Lockley, S. W., Skene, D. J., Butler, L. J., & Arendt, J. (1999). Sleep and activity rhythms are related to circadian phase in the blind. *Sleep, 22,* 616-623.

Longin, E., Dimitriadis, C., Shazi, S., Gerstner, T., Lenz, T., & König, S. (2009). Autonomic nervous system function in infants and adolescents: impact of autonomic tests on heart rate variability. *Pediatric cardiology*, *30*(3), 311-324. doi:10.1007/s00246-008-9327-8

Loredo, J. S., Nelesen, R., Ancoli-Israel, S., & Dimsdale, J. E. (2004). Sleep quality and blood pressure dipping in normal adults. *Sleep, 27,* 1097-1103.

Lorena, S. L., Figueiredo, M. J., Almeida, J. R., & Mesquita, M. A. (2002). Autonomic function in patients with functional dyspepsia assessed by 24-hour heart rate variability. *Dig Dis Sci, 47,* 27-31.

Low, C. A., Salomon, K., & Matthews, K. A. (2009). Chronic life stress, cardiovascular reactivity, and subclinical cardiovascular disease in adolescents. *Psychosomatic Medicine, 71,* 927–931.

Lu, G., Yang, F., Taylor, J.A., & Stein, J. F. (2009). A comparison of photoplethysmography and ECG recording to analyse heart rate variability in healthy subjects. *J Med Eng Tech, 33,* 634-641.

Lumeng, J. C., Somashekar, D., Appugliese, D., Kaciroti, N., Corwyn, R. F., & Bradley, R. H. (2007). Shorter sleep duration is associated with increased risk of being overweight at ages 9 to 12 years. *Pediatrics, 120,* 1020-1029.

Lusardi, P., Mugellini, A., Preti, P., Zoppi, A., Derosa, G., & Fogari, R. (1996). Effects of a restricted sleep regimen on ambulatory blood pressure monitoring in normotensive subjects. *American Journal of Hypertension 9, 503-505.*

Lyytikainen, P., Lallukka, T., Lahelma, E., & Rahkonen, O. (2011). Sleep problems and major weight gain: A follow-up study. *Int J Obes*, *35,* 109–114.

MacKinnon, D. P., & Dwyer, J. H. (1993). Estimating mediated effects in prevention studies. *Evaluation Review*, *17*, 144-158.

MacKinnon, D. P., Warsi, G., & Dwyer, J. H. (1995). A simulation study of mediated effect measures. *Multivariate Behavioral Research*, *30*, 41-62.

Malik, M., & Camm, A. J. (1995). *Heart rate variability*. Armonk, NY: Futura Publishing Company.

Malliani, A., Pagani, M., Lombardi, F., & Cerutti, S. (1991). Cardiovascular neural regulation explored in the frequency domain. *Circulation 84,* 482–492.

Malliani, A., Pagani, M., & Lombardi, F. (1994). Physiology and clinical implications of variability of cardiovascular parameters with focus on heart rate and blood pressure. *American Journal of Cardiology, 73,* 3C-9C.

Malpas, S., & Purdie, G. L. (1990). Circadian variation of heart rate variability. *Cardiovasc Res, 24*, 210-213. doi: 10.1093/cvr/24.3.210

Marshall, N. S., Glozier, N., & Grunstein, R. R. (2008). Is sleep duration related to obesity? A critical review of the epidemiological evidence. *Sleep Medicine Reviews, 12,* 289–298. doi:10.1016/j.smrv.2008.03.001

Marshall, W. A., & Tanner, J. M. (1969). Variations in pattern of pubertal changes in girls. *Arch Dis Child, 44,* 291–303.

Marshall, W. A., & Tanner, J. M. (1970). Variations in the pattern of pubertal changes in boys. *Arch Dis Child*, *45*, 13–23.

Martini, G., Riva, P., Rabbia, F., Molini, V., Ferrero, G. B., Cerutti, F., Carra, R., et al. (2001). Heart rate variability in childhood obesity. *Clinical autonomic research : Official journal of the Clinical Autonomic Research Society*, *11(2),* 87-91. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11570608

Mase, A., Nagae, D., Ito, N., & Komada, Y. (2010). Non-contact and non-invasive stress evaluation using microwave reflectometry [Abstract]. Electro Adv Applicat (ICEAA) 144.

Masi, C. M., Hawkley, L. C., Rickett, E. M., & Cacioppo, J. T. (2007). Respiratory sinus arrhythmia and diseases of aging: Obesity, diabetes mellitus, and hypertension. *Biol Psychol*, *74*, 212–223.

Massin, M. M., Derkenne, B., & Tallsund, M. (1999). Cardiac autonomic dysfunction in diabetic children. *Diabetes*, *22*(11).

Massin, M. M., Maeyns, K., Withofs, N., Ravet, F., & Gérard, P. (2000). Circadian rhythm of heart rate and heart rate variability. *Arch Dis Child, 83,* 179-182.

Massin, M, & von Bernuth, G. (1997). Normal ranges of heart rate variability during infancy and childhood. *Pediatric cardiology*, *18*, 297-302.

Massin, M, & von Bernuth, G. (1998). Clinical and haemodynamic correlates of heart rate variability in children with congenital heart disease. *European journal of pediatrics*, *157*(12), 967-971.

Matthews, S. C., Paulus, M. P., Simmons, A. N., Nelesen, R. A., & Dimsdale, J. E. (2004). Functional subdivisions within anterior cingulate cortex and their relationship to autonomic nervous system function. *Neuroimage 22*, 1151–1156.

Matthews, K. A., Salomon, K., Kenyon, K., & Allen, M. T. (2002). Stability of children's and adolescents' hemodynamic responses to psychological challenge: A three-year longitudinal study of a multiethnic cohort of boys and girls *Psychophysiology*, *39,* 826 –34.

Mazurak, N., Stein, J., Kipphan, S., Muth, E. R., Teufel, M., Zipfel, S., & Enck, P. (2011). Heart rate variability in anorexia nervosa and the irritable bowel syndrome. *Neurogastroenterol Motil, 23*, e470–e478.

McCabe, P. M., & Porges, S.W., & Carter, C.S. (1981). Heart period variability during estrogen exposure and withdrawal in female rats. *Physiol Behav, 26,* 535–538.

McCraty, R., Atkinson, M., & Tomasino, D. (1999). The impact of an emotional self-management skills course on psychosocial functioning and autonomic recovery to stress in middle school children. *Integrative Psychological and Behavioral Sci., 34*(4), 246-268.

McEwen, B. S. (2002). Sex, stress and the hippocampus: Allostasis, allostatic load and the aging process. *Neurobiology of Aging, 23*, 921-939.

McEwen, B. S. (2006). Sleep deprivation as a neurobiologic and physiologic stressor: Allostasis and allostatic load. *Metabolism, 55,* S20-S23.

McEwen, B. S., & Gianaros, P. J. (2010). Central role of the brain in stress and adaptation: Links to socioeconomic status, health, and disease. *Ann. N. Y. Acad. Sci., 1186*, 190-222.

McMillan, D. E. (2002). Interpreting heart rate variability sleep/wake patterns in cardiac patients. *J Cardiovasc Nurs., 17,* 69-81.

Melanson, E. L., & Freedson, P. S. (2001). The effect of endurance training on resting heart rate variability in sedentary adult males. *Eur J Appl Physiol, 85*, 442–449.

Messerli, F. H., Nunez, B. D., Ventura, H. O., & Snyder, D. W. (1987). Overweight and sudden death. Increased ventricular ectopy in cardiopathy of obesity. *Archives of Internal Medicine, 147,* 1725-1728.

Mezzacappa, E., Tremblay, R. E., Kindlon, D., Saul, J. P., Arseneault, L., Séguin, J. R., Pihl, R. O., & Earls, F. (1997). Anxiety, antisocial behavior and heart rate

regulation in adolescent males. *Journal of Child Psychology & Psychiatry, 38,* 457-469.

Molgaard, H., Sorensen, K. E., & Bjerregaard, P. (1991). Circadian variation and influence of risk factors on heart rate variability in healthy subjects. *The American Journal of Cardiology, 68,* 777-784.

Montano, N., Tobaldini, E., & Porta, A. (2012). *The autonomic nervous system*. In Stress Challenges and Immunity in Space: Chouker, A, (Ed.). Heidelberg, Germany: Springer.

Moore, M., Allison, D., & Rosen, C. L. (2006). A review of pediatric nonrespiratory sleep disorders. *Chest, 130,* 1252-1262.

Moore, M., Kirchner, H. L., Drotar, D., Johnson, N., Rosen, C., & Redline, S. (2011). Correlates of adolescent sleep time and variability in sleep time: The role of individual and health related characteristics. *Sleep Med*, *12,* 239-245.

Morris, N. M., & Udry, J. R. (1980). Validation of a self-administered instrument to assess stage of adolescent development. *J Youth Adolesc, 9,* 271-280.

Mortara, D. W. (1977). *Digital filters for ECG signals*. In: Computers in Cardiology New York, NY: Institute of Electrical and Electronics Engineers, pp. 511–514.

Must, A., & Anderson, S. E. (2000). Establishing a standard definition for childhood overweight and obesity worldwide: International survey. *Brit Med J*, 320, 1240-1245.

Must, A., & Strauss, R.S. (1999). Risks and consequences of childhood and adolescent obesity. *International Journal of Obesity Related to Metabolic Disorders, 23,* S2-S11.

Myers, B. L., & Badia, P. (1995). Changes in circadian rhythms and sleep quality with aging: Mechanisms and interventions. *Neurosci Biobehav Rev., 19*, 553–571.

Nagai, N., Matsumoto, T., Kita, H., & Moritani, T. (2003). Autonomic nervous system activity and the state and development of obesity in Japanese school children. *Obesity Research*, *11*(1), 25-32. doi:10.1038/oby.2003.6

Nagai, N. & Moritani, T. (2004). Effect of physical activity on autonomic nervous system function in lean and obese children. *Journal of the International Association for the Study of Obesity*, *28*(1), 27-33. doi:10.1038/sj.ijo.0802470

Napadow, V., Dhond, R., Conti, G., Makris, N., Brown, E. N., & Barbieri, R. (2008). Brain correlates of autonomic modulation: Combining heart rate variability with fMRI. *Neuroimage, 42,* 169-177.

Narkiewicz, K., & Somers, V. K. (2003). Sympathetic nerve activity in obstructive sleep apnoea. *Acta Physiologica Scandinavica*, *177,* 385-390.

National Sleep Foundation. 2004 "*Sleep in America*" Poll. Washington, DC: National Sleep Foundation; 2004.

National Sleep Foundation. 2006 "*Sleep in America*" Poll. Washington, DC: National Sleep Foundation; 2008.

Netherton, C., Goodyer, I., Tamplin, A., & Herbert, J. (2004). Salivary cortisol and dehydroepiandrosterone in relation to puberty and gender. *Psychoneuroendocrinology, 29*, 125–140.

Nielsen, L. S., Danielsen, K. V., & Sørensen, T. I. A. (2011). Short sleep duration as a possible cause of obesity: Critical analysis of the epidemiological evidence. *Obesity Reviews*, *12*, 78-92.

Niskanen, J. P., Tarvainen, M. P., Rantaaho, P. O., & Karjalainen, P. A. (2004). Software for advanced HRV analysis. *Comput Methods Programs Biomed, 76,* 73-81.

Nugent, A. C., Bain, E. E., Thayer, J. F., Sollers, J. J., & Drevets, W. C. (2011). Heart rate variability during motor and cognitive tasks in females with major depressive disorder. *Psychiatry Res, 191*, 1-8.

Nunan, D., Sandercock, G. R. H., & Brodie, D. A. (2010). A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing and clinical electrophysiology, 33*, 1407-1417.

O'Connor, M. F., Gundel, H., McRae, K., & Lane, R. D. (2007). Baseline vagal tone predicts BOLD response during elicitation of grief. *Neuropsychopharmacology, 32*, 2184–2189.

Ogden, C. L, Carroll, M. D., Curtin, L. R., Lamb, M. M., & Flegal, K. M. (2010). Prevalence of high body mass index in US children and adolescents, 2007-2008. *JAMA, 303*, 242-249.

Ogden, C. L., Carroll, M. D., Kit, B. K., & Flegal, K. M. (2012). Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010 . *Jama, 307*, 483–490.

Ogden, C. L., Kuczmarski, R. J., Flegal, K. M., Mei, Z., Guo, S., Wei, R., et al. (2002). Growth charts for the United States: Improvements to the 1977 National Center for Health Statistics Version. *Pediatrics*, *109*, 45-60.

Ohkubo, T., Hozawa, A., Yamaguchi, J., Kikuya, M., Ohmori, K., Michimata, M. et al. (2002). Prognostic significance of the nocturnal decline in blood pressure in

individual with and without high 24-h blood pressure: The Ohasama study. *Hypertension, 20,* 2183-2189.

Olds, T., Blunden, S., Dollman, J., & Maher, C. (2010). Day type and the relationship between weight status and sleep duration in children and adolescents. *Aust N Z J Public Health, 34,* 165-71.

Olds, T. S., Maher, C. A., & Matricciani, L. (2011). Sleep duration or bedtime? Exploring the relationship between sleep habits and weight status and activity patterns. *Sleep, 34,* 1299-1307.

Olds, T., Tomkinson, G., Maher, C., & Ferrar, K. (2010). Trends in the prevalence of childhood overweight and obesity in Australia between 1985 and 2008. *Int J Obes*, *34*, 57-66.

Ordaz, S., & Luna, B. (2012). Sex differences in physiological reactivity to acute psychosocial stress in adolescence. *Psychoneuroendocrinology*. Advance online publication doi:10.1016/j.psyneuen.2012.01.002

Owens, J. A., Spirito, A., & McGuinn, M. (2000). The children's sleep habits Questionnaire (CSHQ): Psychometric properties of a survey instrument for school-aged children. *Sleep, 23,* 1043-1051.

Pagani, M., Montano, N., Porta, A., Malliani, A., Abboud, F., Nirkett, C., & Somers, V. K. (1997). Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation, 95,* 1441– 8.

Palatini, P., & Julius, S. (2009). The role of cardiac autonomic function in hypertension and cardiovascular disease. *Curr Hypertens Rep, 11*, 199–205.

Pan, J., & Tompkins, W. J. (1985). A real-time QRS detection algorithm. *IEEE Trans Biomed Eng, 32*, 230-236.

Pan, X., & Hussain, M. M. (2009). Clock is important for food and circadian regulation of macronutrient absorption in mice. *J Lipid Res, 50,* 1800–1813.

Pannain, S., & Van Cauter, E. (2008). Sleep loss, obesity and diabetes: Prevalence, association and emerging evidence for causation. *Obesity Metab, 4*, 28–41.

Parati, G., Saul, J. P., Di Rienzo, M., & Mancia, G. (1995). Spectral analysis of blood pressure and heart rate variability in evaluating cardiovascular regulation: A critical appraisal. *Hypertension, 25,* 1276-1286.

Park, M. K., & Menard, S. M. (1998). Accuracy of blood pressure measurement by the Dinamap monitor in infants and children. *Pediatrics*, *8,* 308-18

Pateyjohns, I. R., Brinkworth, G. D., Buckley, J. D., Noakes, M., & Clifton, P. M. (2006). Comparison of three bioelectrical impedance methods with DXA in overweight and obese men. *Obesity (Silver Spring)*, *14*, 2064–207.

Pincus, S. M. (1995). Approximate entropy (ApEn) as a complexity measure. *Chaos, 5,* 110-118.

Pinna, G., Maestri, R., Torunski, A., Danilowicz-Szymanowicz, L., Szwoch, M., La Rovere, M. T., & Raczak, G. (2007). Heart rate variability measures: A fresh look at reliability. *Clin Sci (Lond), 113*, 131–140.

Pipberger, H. V., Stallman, F. W., & Berson, A. S. (1962). Automatic analysis of the P-QRS-T complex of the electrocardiogram by digital computer. *Ann Intern Med, 57*, 776–787.

Poliakova, N., Després, J-P., Bergeron, J., Alméras, N., Tremblay, A., & Poirier, P. (2012). Influence of obesity indices, metabolic parameters and age on cardiac autonomic function in abdominally obese men. *Metabolism*. Advance online publication doi:10.1016/j.metabol.2012.02.006

Pomeranz, B., Macaulay, R. J., Caudill, M. A., Kutz, I., Adam, D., Gordon, D., et al. (1985). Assessment of autonomic function in humans by heart rate spectral analysis. *American Journal of Physiology- Heart and Circulatory Physiology, 248*, 151-153.

Porta, A., Guzzetti, S., Furlan, R., Gnecchi-Ruscone, T., Montano, N., & Malliani, A. (2007). Complexity and nonlinearity in short-term heart period variability: Comparison of methods based on local nonlinear prediction. *IEEE Trans Biomed Eng, 54,* 94–106.

Porta, A., Guzzetti, S., Montano, N., Furlan, R., Pagani, M., Malliani, A., & Cerutti, S. (2001). Entropy, entropy rate, and pattern classification as tools to typify complexity in short heart period variability series. *IEEE Trans Biomed Eng, 48,* 1282–1291.

Pryor, T. A., Russell, R., Budkin, A., & Price, W. G. (1969). Electrocardiographic interpretation by computer. *Comput Biomed Res, 2,* 537– 548.

Rabbia, F., Silke, B., Conterno, A., Grosso, T., De Vito, B., Rabbone I., et al. (2003). Assessment of cardiac autonomic modulation during adolescent obesity. *Obes Res, 11*, 541-548.

Raju, K. N. V., Choudhary, N., Gulati, S., Kabra, M., Jaryal, A. K., Deepak, K. K., & Pandey, R. M. (2012). Comparison of heart rate variability among children with

well controlled versus refractory epilepsy: A cross-sectional study. *Epilepsy research*, 3-6. Advance online publication. doi:10.1016/j.eplepsyres.2012.03.005

Rao, M. N., Blackwell, T., Redline, S., Stefanick, M. L., Ancoli-Israel, S., & Stone, K. L. (2009). Association between sleep architecture and measures of body composition. *Sleep, 32,* 483-490.

Rayner, D. V., & Trayhurn, P. (2001). Regulation of leptin production: sympathetic nervous system interactions. *J Mol Med, 79*, 8–20.

Reed, K. E., Warburton, D. E. R., Whitney, C. L., & McKay, H. A. (2006). Differences in heart rate variability between Asian and Caucasian children living in the same Canadian community. *Applied Physiology, Nutrition, and Metabolism*, *31*(3), 277-282. NRC Research Press. doi:10.1139/h05-015

Reilly, J. J., Armstrong, J., Dorosty, A. R., Emmett, P. M., Ness, A., Rogers, I., et al. (2005). Early life risk factors for obesity in childhood: Cohort study. *British Medical Journal, 330,* 1357-1364.

Richman, J. S., & Moorman, J. R. (2000). Physiological time-series analysis using approximate entropy and sample entropy. *Am J Physiol Heart Circ Physiol, 278,* H2039-H2049.

Riva, P., Martini, G., Rabbia, F., Milan, A., Paglieri, C., Chiandussi, L., & Veglio, F. (2001). Obesity and autonomic function in adolescence. *Clin Exp Hypertens, 23,* 57-67.

Roberts, R. E., Roberts, C. R., & Chan, W. (2006). Ethnic differences in symptoms of insomnia among adolescents. *Sleep, 29(3),* 359–365.

Rodríguez-Colón, S. M., Bixler, E. O., Li, X., Vgontzas, A. N., & Liao, D. (2011). Obesity is associated with impaired cardiac autonomic modulation in children. *Int J Pediatr Obes, 6,* 128-134.

Rodríguez-Colón, S. M., He, F., Shaffer, M. L., Li, X., Vgontzas, A. N., Bixler, E. O., Wu, R., et al. (2011). Insomnia Symptoms and Sleep Duration Are Associated with Impaired Cardiac Autonomic Modulation in Children. *Neuroscience & Medicine, 02(03),* 288-294. doi:10.4236/nm.2011.23037

Roeser, K., Obergfell, F., Meule, A., Vogele, C., Schlarb, A. A., & Kubler, A. (2012). Of larks and hearts-morningness/eveningness, heart rate variability and cardiovascular stress response at different times of day. *Physiology & Behavior, 106*, 151-157.

Rogol, A. D., Clark, P. A., & Roemmich, J. N. (2000). Growth and pubertal development in children and adolescents: Effects of diet and physical activity. *Am J Clin Nutr, 72*, 521s-528s.

Romhilt, D. W., & Estes, E. H. Jr. (1968). A point-score system for the ECG diagnosis of left ventricular hypertrophy. *Am Heart J, 75,* 752– 758.

Rueggeberg, R., Wrosch, C., & Miller, G. E. (2012). Sleep duration buffers diurnal cortisol increases in older adulthood. *Psychoneuroendocrinology*, *37*, 1029-1038.

Rydberg, A., Rask, P., Hörnsten, R., & Teien, D. (2004). Heart rate variability in children with Fontan circulation. *Pediatric cardiology, 25(4),* 365-9. doi:10.1007/s00246-003-0552-x.

Sakurai, T. (2007). The neural circuit of orexin (hypocretin): Maintaining sleep and wakefulness. *Nat Rev Neurosci., 8,* 171–181.

Salameh, A., Gebauer, R. A., Grollmuss, O., Vit, P., Reich, O., & Janousek, J. (2008). Normal limits for heart rate as established using 24-hour ambulatory electrocardiography in children and adolescents. *Cardiol Young, 18*, 467–472.

Sallis, J. F., Buono, M. J., Roby, J. J., Micale, F. G., & Nelson, J. A. (1993). Seven-day recall and other physical activity self-reports in children and adolescents. *Med Sci Sports Exerc., 25,* 99–108.

Salo, M. A., Huikuri, H. V., & Seppanen, T. (2001). Ectopic beats in heart rate variability analysis: Effects of editing on time and frequency domain measures. Ann *Noninvasive Electrocardiol., 6,* 5–17.

Sandercock, G. R., Bromley, P., & Brodie, D. A. (2004). Reliability of three commercially available heart rate variability instruments using short-term (5-min) recordings. *Clin Physiol Funct Imaging, 24,* 359–367

Sandercock, G. R. H., Bromley, P. D., & Brodie, D. A. (2005). The reliability of short-term measurements of heart rate variability. *Int J Cardiol, 103*, 238–247.

Sandercock, G. R. H., Shelton, C., & Brodie, D. A. (2003). Heart rate variability instrumentation: Agreement and reliability. *J Physiol, 552P*, C34.

Scheer, F. A., Hilton, M. F., Mantzoros, C. S., & Shea, S. A. (2009). Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci U S A, 106,* 4453–4458.

Schmid, S. M., Hallschmid, M., Jauch-Chara, K., Born, J., & Schultes, B. (2008). A single night of sleep deprivation increases ghrelin levels and feelings of hunger in normal-weight healthy men. J Sleep Res, *17*, 331–334.

Schroeder, E. B., Duanping, L., Chambless, L. E., Prineas, R. J., Evans, G.W., & Heiss, G. (2003). Hypertension, blood pressure, and heart rate variability. The atherosclerosis risk in communities (ARIC) study. *Hypertension, 42,* 1106–1111.

Sforza, E., Chapotot, F., Pigeau, R., Naitoh, P., & Buguet, A. (2004). Effects of sleep deprivation on spontaneous arousals in humans. Sleep, *27,* 1068-1075.

Sforza, E., Pichot, V., Cervena, K., Barthelemy, J. C., & Roche, F. (2007). Cardiac variability and heart-rate increment as a marker of sleep fragmentation in patients with a sleep disorder: A preliminary study. *Sleep, 30,* 43-51.

Sgoifo, A., Buwalda, B., Roos, M., Costoli, T., Merati, G. & Meerlo, P. (2006). Effects of sleep deprivation on cardiac autonomic and pituitary-adrenocortical stress reactivity in rats. *Psychoneuroendocrinology, 31,* 197-208.

Shannon, D. C., Carley, D. W., & Benson, H. (1987). Aging of modulation of heart rate. *Am J Physiol, 253,* H874-H877.

Sharma, R. K., Balhara, Y. P. S., Sagar, R., Deepak, K. K., & Mehta, M. (2011). Heart rate variability study of childhood anxiety disorders. *J Cardiovasc Dis Res.,* 2(2), 115-122. doi:10.4103/0975-3583.83040

Shields, M. (2006). Overweight and obesity among children and youth. *Health Rep, 17,* 27–42.

Shrout, P. E., & Fleiss, J. L. (1979). Intraclass correlations: Uses in assessing rater reliability. *Psychol Bull, 86,* 420-428.

Silvetti, M. S., Drago, F., & Ragonese, P. (2001). Heart rate variability in healthy children and adolescents is partially related to age and gender. *International*

Journal of cardiology, *81,* 169-174. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11744133

Sinnreich, R., Kark, J. D., Friedlander, Y., Sapoznikov, D., & Luria, M. H. (1998). Five minutes recordings of heart rate variability for population studies: Repeatability and age-sex characteristics. *Heart, 80,* 156–162.

Smith, C. S., Reilly, C., & Midkiff, K. J. (1989). Evaluation of three circadian rhythm questionnaires with suggestions for an improved measure of morningness. *Applied Psychology, 74,* 728-738.

Snell, E. K., Adam, E. K., & Duncan, G. J. (2007). Sleep and the body mass index and overweight status of children and adolescents. *Child Development, 78,* 309-323.

Snitker, S., Macdonald, I., Ravussin, E., & Astrup, A. (2000). The sympathetic nervous system and obesity: Role in aetiology and treatment. *Obesity Reviews, 1*, 5-15.

Sobel, M. E. (1982). Asymptotic intervals for indirect effects in structural equations models. In S. Leinhart (Ed.), *Sociological methodology,* (pp.290-312). San Francisco: Jossey-Bass.

Soergel, M. (1999). Development of normative ambulatory blood pressure data in children. *Arbeitsgruppe padiatrische Hypertonie. Blood Press Monit., 4,* 121-126.

Somers, V. K., Dyken, M. E., Clary, M.P., & Abboud, F. M. (1995) Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest, 96,* 1897e904.

Spear, L. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev., 24*, 417-463.

Spiegel, K., Knutson, K., Leproult, R., Tasali, E., & Van Cauter, E. (2005). Sleep loss: a novel risk factor for insulin resistance and Type 2 diabetes. *Journal of Applied*

Physiology, 99, 2008-2019.

Spiegel, K., Leproult, R., L'Hermite-Baleriauz, M., Copinschi, G., Penev, P. D., & Van Cauter, E. (2004). Leptin levels are dependent on sleep duration: Relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. *The Journal of Clinical Endocrinology & Metabolism, 89*, 5762-5771.

Spiegel, K., Leproult, R. & Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. *Lancet, 354*, 1435-1439.

Spiers, J. P., Silke, B., McDermott, U., Shanks, R.G., & Harron, D. W. (1993). Time and frequency domain assessment of heart rate variability: A theoretical and clinical appreciation. *Clinical Autonomic Research, 3,* 145–158.

Stamatakis, K., & Punjabi, N. M. (2010). Effects of sleep fragmentation on glucose metabolism in normal subjects. *Chest*, *137,* 95-101.

Statistics Canada (2009). *Table 105-0506 - Measured youth body mass index (BMI), by age group and sex, household population aged 12 to 17 excluding pregnant females, Canada (excluding territories), occasional (number unless otherwise noted),* CANSIM (database).

Steckelings, U. M., Rompe, F., Kaschina, E., & Unger, T. (2009). The evolving story of the RAAS in hypertension, diabetes and CV disease: Moving from macrovascular to microvascular targets. *Fundam Clin Pharmacol., 23,* 693-703.

Stein, P. K., & Kleiger, P. K. (1999). Insights from the study of heart rate variability. *Annual Review of Medicine, 50,* 249-261.

Stein, P. K., Kleiger, R. E, & Rottman, J. N. (1997). Differing effects of age on heart rate variability in men and women. *Am. J. Cardiol., 80,* 302-305.

Storfer-Isser, A., Patel, S. R., Babineau, D. C., & Redline, S. (2012). Relation between sleep duration and BMI varies by age and sex in youth age 8–19. *Pediatric Obesity* , *7*, 53–64.

Strine , T. W., & Chapman, D. P. (2005). Associations of frequent sleep insufficiency with health-related quality of life and health behaviors. *Sleep Med, 6,* 23-27.

Tasali, E., Leproult, R., Ehrmann, D. A., & Van Cauter, E. (2008). Slow-wave sleep and the risk of type 2 diabetes in humans. *Proc Natl Acad Sci U S A,105,* 1044-1049.

Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation and clinical use. *Circulation 93,* 1043–1065.

Taveras, E. M., Rifas-Shiman, S. L., Oken, E., Gunderson, E. P., & Gillman, M. W. (2008). Short sleep duration in infancy and risk of childhood overweight. *Archives of Pediatrics and Adolescent Medicine, 162,* 305-311.

Taylor, J. A., Carr, D. L., Myers, C. W., & Eckberg, D. L. (1998). Mechanisms underlying very-low-frequency RR-interval oscillations in humans. *Circulation*, *98*, 547–555.

Thayer, J. F., & Lane, R. D. (2007). The role of vagal function in the risk for cardiovascular disease and mortality. *Biol Psycho, 74*, 224-242.

Thayer, J. F., & Sternberg, E. (2006). Beyond heart rate variability: Vagal regulation of allostatic systems. *Ann N Y Acad Sci., 1088*, 361–372. doi: 10.1196/annals.1366.014

Theorell-Haglöw, J., Berne, C., Janson, C., Sahlin, C., & Lindberg, E. (2010).

Associations between short sleep duration and central obesity in women. *Sleep*, *33,* 593-598.

Thomas, R. J., Mietus, J. E., Peng, C. K., Gilmartin, G., Daly, R. W., Goldberger, A. L., et al. (2007). Differentiating obstructive from central and complex sleep apnea using an automated electrocardiogram-based method. *Sleep, 30,* 1756e69.

Timonen, K. L., Vanninen, E., de Hartog, J., Ibald-Mulli, A., Brunekreef, B., Gold, D. R., Heinrich, J., Hoek, G., Lanki, T., Peters, A., Tarkiainen, T., Tiittanen, P., Kreyling, W., & Pekkanen, J. (2006). Effects of ultrafine and fine particulate and gaseous air pollution on cardiac autonomic control in subjects with coronary artery disease: The ULTRA study. *J Expo Sci Environ Epidemiol, 16*, 332-341.

Tochikubo, O., Ikeda, A., Miyajima, E., & Ishii, M. (1996). Effects of Insufficient Sleep on Blood Pressure Monitored by a New Multibiomedical Recorder. *Hypertension, 27*, 1318-1324.

Tonhajzerova, I., Ondrejka, I., Javorka, K., Turianikova, Z., Farsky, I., & Javorka, M. (2010). Cardiac autonomic regulation is impaired in girls with major depression. *Progress in neuro-psychopharmacology & biological psychiatry*, *34*(4), 613-8. doi:10.1016/j.pnpbp.2010.02.023

Trakada, G., Chrousos, G., Pejovic, S., & Vgontzas, A. (2007). Sleep apnea and its association with the stress system, inflammation, insulin resistance and visceral obesity. *Sleep Med Clin* , *2*, 251-261.

Tremblay, M. S., Katzmarzyk, P. T., & Willms, J. D. (2002). Temporal trends in overweight and obesity in Canada, 1981-1996. *Int J Obes, 26*, 538-543.

Trinder, J., Kleiman, J., Carrington, M., Smith, S., Breen, S., Tan, N. et al. (2001). Autonomic activity during human sleep as a function of time and sleep stage. *Journal of Sleep Research, 10*, 253-264.

Tsuji, H., Venditti, F. J., Manders, E. S., Evans, J. C., Larson, M. G., Feldman, C. L., & Levy, D. (1996). Determinants of heart rate variability. *Journal of the American College of Cardiology*, *28*(6), 1539-1546. doi:10.1016/S0735-1097(96)00342-7

Turek, F. W., Joshu, C., Kohsaka, A., Lin, E., Ivanova, G., McDearmon, E. *et al. (2005)* Obesity and metabolic syndrome in circadian Clock mutant mice. *Science, 308,* 1043–1045.

Umetani, K., Singer, D. H., McCraty, R., & Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *Journal of the American College of Cardiology*, *31*(3), 593-601. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9502641

Unalacak, M., Aydin, M., Ermis, B., Ozeren, A., Sogut, A., Demirel, F., & Unluoglu, I. (2004). Assessment of cardiac autonomic regulation in children with monosymptomatic nocturnal enuresis by analysis of heart rate variability. *The Tohoku journal of experimental medicine*, *204(1),* 63-9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15329464

van Bemmel, J. H., Zywietz, C., & Kors, J. A. (1990). Signal analysis for ECG interpretation. *Methods Inform Med, 29,* 317–329.

Van Cauter, E., Holmback, U., Knutson, K., Leproult, R., Miller, A., Nedeltcheva, A., Pannain, S., Penev, P., Tasali, E., & Spiegel, K., (2007). Impact of sleep and sleep loss on neuroendocrine and metabolic function. *Horm. Res., 67,* (Suppl. 1), 2–9.

Van Cauter, E., Polonsky, K. S, & Scheen, A. J. (1997). Roles of circadian rhythmic- ity and sleep in human glucose regulation. *Endocr Rev, 18*, 716–738.

van den Berg, J. F., Neven, A. K., Tulen, J. H. M., Hofman, A., Witteman, J. C. M., Miedema, H. M. E. *et al.(2008).* Actigraphic sleep duration and fragmentation are related to obesity in the elderly: The Rotterdam Study. *Int J Obes*, *32,* 1083–1090.

vandenTop, M., Lee, K., Whyment, A. D., Blanks, A. M., & Spanswick, D. (2004). Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. *Nature Neurosci*, *7*, 493-494.

van Eekelen, A. P. J., Varkevisser, M., & Kerkhof, G. A.(2003). Cardiac autonomic activity during human sleep: Analysis of sleep stages and sleep cycles. *Biological Rhytm Research, 34,* 493-502.

Van Egeren, L., Haynes, S. N., Franzen, M., & Hamilton, J. (1983). Presleep cognitions and attributions in sleep-onset insomnia. *Journal of Behavioral Medicine, 6,* 217–232.

Verdecchia, P., Schillaci, G., Guerrieri, M., Gatteschi, C., Benemio, G., Boldrini, F. et al. (1990). Circadian blood pressure changes and left ventricular hypertrophy in essential hypertension. *Circulation, Journal of the American Heart Association, 81,* 528-536.

Vgontzas, A. N., Zoumakis, M., Bixler, E. O., Lin, H.-M., Prolo, P., Vela-Bueno, A., Kales, A. & Chrousos, G. P. (2003). Impaired nighttime sleep in healthy old versus young adults is associated with elevated plasma Interleukin-6 and

cortisol levels: Physiologic and therapeutic implications. *J Clin Endocrinol Metabo, 88*, 2087-2095.

Vinet, A, Beck, L., Nottin, S., & Obert, P. (2005). Effect of intensive training on heart rate variability in prepubertal swimmers. *European journal of clinical investigation*, 35(10), 610-4. doi:10.1111/j.1365-2362.2005.01557.x

Voss, A., Schulz, S., Schroeder, R., Baumert, M., & Caminal, P. (2009). Methods derived from nonlinear dynamics for analysing heart rate variability. *Philos Transact A Math Phys Eng Sci, 367*, 277–296.

Wallis, L. A., Healy, M., Undy, M. B., & Maconochie, I. (2005). Age related reference ranges for respiration rate and heart rate from 4 to 16 years. *Arch Dis Child, 90,* 1117-1121.

Walter, L. M., Foster, A. M., Patterson, R. R., Anderson, V., Davey, M. J., Nixon, G. M., Trinder, J., et al. (2009). Cardiovascular variability during periodic leg movements in sleep in children. *Sleep*, *32*(8), 1093-9. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2717200&tool=pmcentrez&rendertype=abstract

Wang, G., & Dietz, W. (2002). Economic burden of obesity in youths aged 6 to 17 years: 1979-1999. *Pediatrics, 109*, 1-6.

Wang, X., Thayer, J. F., Treiber, F., & Snieder, H. (2005). Ethnic differences and heritability of heart rate variability in African- and European American youth. *The American journal of cardiology*, *96*(8), 1166-72. doi:10.1016/j.amjcard.2005.06.050

Wawryk, A. M., Bates, D. J., & Couper, J. J. (1997). Power spectral analysis of heart rate variability in children and adolescents with IDDM. *Diabetes care*, *20*(9), 1416-21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9283789

Weaver, E. M., Kapur, V., &Yueh, B. (2004). Polysomnographyvs self-reported measures in patients with sleep apnea. Archives of Otolaryngology - *Head & Neck Surgery*, *130,* 453-458.

Webber, L. S., Osganian, V., Luepker, R.V., Feldman, H.A., Stone, E.J., Elder, J.P., et al. (1995). Cardiovascular risk factors among third grade children in four regions of the United States: The CATCH study. *Am J Epidemiol, 141*, 428–439.

Wei, C. Y., Chung, T. C., Wu, S. C., Chung, C. F., & Wu, W. P. (2011). The subjective sleep quality and heart rate variability in hemodialysis patients. *Ren Fail., 33*, 109-117.

Welch, P. D. (1967). The use of fast Fourier transforms for the estimation of power spectra: A method based on time averaging over short modified periodograms. *IEEE Trans Audio Electroacoustics, 15*, 70-73.

Welk, G., Wickel, E., Peterson, M., Heitzler, C., Fulton, J., & Potter, L. (2007). Reliability and validity of questions on the youth media campaign longitudinal survey. *Med Sci Sports Exerc,39,* 612–621.

Wheaton, A. G., Perry, G. S., Chapman, D. P., McKnight-Eily, L. R., Presley-Cantrell, L. R., & Croft, J. B. Relationship between body mass index and perceived insufficient sleep among U.S. adults: An analysis of 2008 BRFSS data. *BMC Public Health, 11,* 295-312.

Winsley, R. J., Armstrong, N., Bywater, K., & Fawkner, S. G. (2003). Reliability of heart rate variability measures at rest and during light exercise in children. *British journal of sports medicine*, *37*(6), 550-2. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1724706&tool=pmcentrez&rendertype=abstract

Wolfson, A. R., & Carskadon, M. A. (1998). Sleep schedules and daytime functioning in adolescents. *Child Development, 69,* 875-887.

Wolfson, A. R., Carskadon, M. A., Acebo, C., Seifer, R., Fallone, G., Labyak, S. E., et al. (2003). Evidence for the validity of a sleep habits survey for adolescents. *Sleep, 2*, 213-216.

Wrosch, C., Miller, G. E., Lupien, S., & Pruessner, J. C. (2008). Diurnal cortisol secretion and 2-year changes in older adults' physical symptoms: The moderating roles of negative affect and sleep. *Health Psychology, 27*(6), 685-693. doi:10.1037/0278-6133.27.6.685

Xia, R, Odemuyiwa, O., Gill, J., Malik, M., & Camm, A. J. (1993). Influence of recognition errors of computerised analysis of 24-hour electrocardiograms on the measurement of spectral components of heart rate variability. *Int J Biomed Comput,32,* 223-23.

Yamasaki, Y., Kodama, M., & Matsuhisa, M. (1996). Diurnal heart rate variability in healthy subjects: Effects of aging and sex differences. *Am J Physiol., 271,* 303–310.

Yang, T. F., Wong, T. T., Chang, K. P., Kwan, S. Y., Kuo, W. Y., Lee, Y. C., & Kuo, T. B. J. (2001). Power spectrum analysis of heart rate variability in children with epilepsy. *Child's Nerv Syst., 17,* 602-606.

Yeragani, V. K., Rao, K. A, Pohl, R., Jampala, V. C., & Balon, R. (2001). Heart rate and QT variability in children with anxiety disorders: a preliminary report. *Depression and anxiety*, *13(2),* 72-7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11301923

Yeragani, V. K., Sobolewski, E., Kay, J., Jampala, V. C., & Igel, G. (1997). Effect of age on long-term heart rate variability. *Cardiovascular research*, *35(1),* 35-42.

Youngstrom, T. G., & Bartness, T. J. (1998). White adipose tissue sympathetic nervous system denervation increases fat pad mass and fat cell number. *Am J Physiol, 275*, (5 Pt 2): R1488–R1493.

Zheng, H., Patterson, L. M., Berthoud, H. R., & Orexin, A. (2005). Projections to the caudal medulla and orexin-induced c-Fos expression, food intake, and autonomic function. *J Comp Neurol, 485,* 127–142.

Zhong, X., Hilton, H. J., Gates, G. J., Jelic, S., Stern, Y., Bartels, M. N., et al. (2005). Increased sympathetic and decreased parasympathetic cardiovascular modulation in normal humans with acute sleep deprivation. *Journal of Applied Physiology, 98,* 2024-2032.