The Canadian Sleep and Circadian Network (CSCN): A Longitudinal Study Identifying Children with Obesity Most at Risk for Obstructive Sleep Apnea and their Neurocognitive, Metabolic, and Cardiovascular Outcomes

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In Canada, child and adolescent obesity represents one of the most common conditions affecting children [1]. Obesity is not only complicated by cardiovascular and metabolic dysfunction, but it is also associated with obstructive sleep apnea (OSA). OSA is characterized by snoring, recurrent partial (hypopneas) or complete obstruction (apneas) of the upper airway, frequently associated with intermittent oxyhemoglobin desaturations, sleep disruption and fragmentation [2]. OSA is often associated with neurocognitive deficits and behavioral problems. Our aim is to identify children with obesity most at risk of OSA and to determine the independent effects of OSA on cardiovascular, metabolic, neurobehavioral and cognitive functions.

Methods:
This is a multi-center, observational, longitudinal study at the Hospital for Sick Children (Toronto) and the Children’s Hospital of Eastern Ontario (Ottawa). Children with obesity scheduled to undergo a clinically indicated polysomnogram (PSG) will be recruited from sleep and STOMP (SickKids Obesity Management Program) clinics. Anthropometric measurements and questionnaires, assessing sleep and neurocognitive functioning, will be completed prior to the PSG. Fasting blood work will be collected and evaluated for cardiovascular and metabolic biomarkers. Subjects diagnosed with OSA will have treatment as per clinical standards and will be asked to return yearly as per standard of clinical care which includes yearly follow up PSGs. At yearly follow up visits, measurements of anthropometry and questionnaires assessing sleep and neurocognitive functioning will be repeated. During these visits, fasting blood work for cardiovascular, metabolic biomarkers will be completed. If patients consent to the genetics sub-study, additional blood samples will only be collected following the baseline sleep study.

Objectives:
The primary objective is to identify anthropometric measurements associated with increased risk of OSA in youth with obesity.

The secondary objectives are:
- To determine independent effects of OSA on cardiovascular, metabolic and cognitive functions in obese youth.
- To evaluate the impact of positive airway pressure (PAP) treatment for OSA on cardiovascular, metabolic and neurocognitive functions in obese youth.

Anticipated Outcome:
We anticipate that the neck to waist ratio will predict children with obesity most at risk for OSA. Cardio-metabolic risk, defined as an elevated Homeostatic model assessment for insulin resistance (HOMA-IR) z-score, elevated C-reactive protein (CRP), liver enzymes and lipid profile, will be higher in youth with obesity and OSA compared to those without OSA. HOMA-IR z-score will be reduced after treatment of OSA in youth with obesity. Neurocognitive function and quality of life are reduced in subjects with obesity and OSA compared to obese youth without OSA. Neurocognitive function and quality of life will improve in subjects with OSA, who are treated with and are adherent to PAP therapy.

1. Introduction and Background
Child and adolescent obesity, defined as a body mass index (BMI) of >95th percentile for age and gender [3], represents one of the most common conditions affecting children in Canada with an obesity rate of 10% in 12-17 year old children, which currently equates to approximately half a million obese children in Canada [4]. Obesity is not only complicated by cardiovascular and metabolic dysfunction, but it is also associated with OSA [5]. OSA is a respiratory disorder of sleep that is caused by upper airway obstruction during sleep, resulting in sleep fragmentation and disturbance in children. OSA occurs in 1-4% of healthy children [6]. The commonest etiological factor for childhood OSA in young children is adenotonsillar hypertrophy and further, an adenotonsillectomy is usually curative. As sleep plays a crucial role in the mental, physical and psychosocial well-being of children, OSA is often associated with neurocognitive deficits and behavioral problems [7, 8] as well as increasing evidence to suggest that OSA may magnify underlying cardiovascular and metabolic risk.

However, in the last decade, the childhood obesity epidemic [9] has significantly changed the scope of paediatric OSA as children with obesity are at high risk for upper airway obstruction [5]. The prevalence of OSA in youth with obesity is between 10-50% [5]. Many children with obesity and OSA in Canada will go undiagnosed, in part due to a lack of paediatric sleep services across Canada [10]. Moreover, treatment strategies such as adenotonsillectomy (AT) are only effective in 40% of youth with obesity and OSA [11-13] as additional factors such as increased parapharyngeal fat, and fatty tongue base may all contribute to OSA. Moreover, it is difficult to predict which individual subject is likely to benefit from an AT. As such, continuous positive airway pressure (CPAP) or Bi-level positive airway pressure (Bi-level PAP) is increasingly used as the first line of treatment for OSA in children with obesity [14]. PAP, although efficacious only has adherence rates around 50-70%.

Co-morbidities associated with OSA include poor quality of life, disruptive and impulsive behavior, inattention, and poorer learning and memory. Further, more recent data in adults suggest that OSA is associated with cardiovascular and metabolic dysfunction; elevated C-reactive protein, hypertension and insulin resistance are more prevalent in those with OSA and may normalize following treatment of OSA [15]. There is a paucity of equivalent longitudinal data in youth with obesity.

Importantly, although OSA is increasingly recognized as a co-morbidity in children with obesity, a paucity of screening tools to diagnose OSA limits both the timely diagnosis and therapeutic interventions for OSA. This is especially relevant in the context that 75% of youth with obesity will seamlessly transition to become obese adults, many with co-existing OSA. Thus, there is an urgent need to screen and identify those youth with obesity most at risk for OSA and its related consequences and to direct targeted interventions at the most vulnerable youth with obesity.

Finally, OSA is a complex chronic condition that is undoubtedly influenced by multiple factors. Accumulating data suggest that there are strong genetic underpinnings for this condition [16]. It has been estimated that approximately 40% of the variance in the apnea hypopnea index (AHI) may be explained by familial factors.

2. Rationale for the Proposed Study
In children with obesity, it is unclear which individual child is at risk for OSA; specifically the severity of BMI does not predict OSA. This is particularly important as adults with OSA have an increased risk for cardiovascular morbidity and mortality. Clinical and anthropometric measures predictive of OSA in children with obesity are not well defined, although data suggests that increased neck and waist circumferences may better predict risk of OSA, particularly neck-to-waist circumference ratio (N/W) [17-19]. Further, the relative causal role of OSA in potentiating cardiovascular, metabolic, and cognitive disturbances in children and youth with obesity has not been well-studied [20]. Moreover, in anticipation of certain new genetic developments in this field, future genetic analysis may help us to identify those subjects at the greatest risk for OSA and the complications of OSA, leading to early interventional strategies.

Thus, large scale studies are necessary in youth with obesity to more clearly identify risk factors associated with OSA and associated cardiovascular, metabolic and neurocognitive consequences in obese youth.

3. Research Objectives and Hypothesis

3.1 Objectives

The primary objective is to identify anthropometric measurements associated with increased risk of OSA in youth with obesity.

The secondary objectives are:

- To determine the independent effects of OSA on cardiovascular, metabolic and cognitive functions in obese youth.
- To evaluate the impact of PAP treatment for OSA on cardiovascular, metabolic and neurocognitive functions in obese youth.

3.2 Hypotheses

Primary Hypothesis

1. Neck-to-waist circumference ratio will predict obese youth at high risk for OSA.

Secondary Hypotheses

2. Homeostatic model assessment of insulin resistance (HOMA-IR) z-score will be higher in obese subjects with OSA compared to obese youth without OSA.

3. HOMA-IR z-score will be lower in obese subjects with OSA treated with and adherent to PAP therapy.

Exploratory Hypotheses

4. Additional markers of cardiometabolic dysfunction, including high sensitivity-CRP, full blood count, liver enzymes, fasting lipid, hemoglobin (HbA1c), vitamin D, and electrolytes will have greater prevalence of abnormalities in subjects with OSA compared to those without, and will improve in those with OSA who are adherent to PAP therapy.
5. Neurobehavioral dysfunction will be higher in subjects with OSA compared to those without OSA, and will improve in those with OSA who are adherent to PAP therapy.

6. Quality of life will be impaired in subjects with OSA compared to those without OSA, and will improve in those with OSA who are adherent to PAP therapy.

7. Physical activity level will be lower in subjects with OSA compared to those without OSA, and will improve in those with OSA who are adherent to PAP therapy.

8. Pulmonary function will be lower in obese subjects with OSA compared to those without OSA.

4. Methodology

4.1 Description
This is a 5-year prospective cohort study examining obese children and youth aged 8-17 years who are scheduled to undergo a clinically indicated baseline PSG to assess for OSA. This study will involve two tertiary care pediatric hospitals in Ontario (SickKids and the Children’s Hospital of Eastern Ontario) which have sleep facilities to undertake full overnight PSG. The study protocols will be identical at both institutions.

4.2 Recruitment at SickKids
Our study population will consist of obese children who have been referred to the sleep clinic at SickKids. Additionally, the principal investigator (Dr. I Narang) also reviews obese children for suspected OSA in the STOMP (SickKids Obesity Management Program) clinic. Eligible children who are scheduled to undergo a clinically indicated PSG will be recruited at the STOMP and sleep clinic, where the PSG will occur within 12 weeks of the clinic visit. During the clinic appointment, the physicians (Principal and Co-Investigators) will determine if the child is eligible to take part in the study. If the child seems to be eligible, the physician who is either the Principal or one of the Co-Investigators will inform the child and family about the study. All eligible subjects will be invited to participate in the study by the research assistant. If the parents have questions that cannot be answered by the research coordinator, these questions will be directed to the primary investigator, Dr. I Narang or co-investigators.

Parents and subjects will be informed that enrolment is voluntary and that they can withdraw from the study at any time and the decision to participate or not will have no bearing on the medical care they receive. The study will be explained verbally according to the information included in the consent forms. Consent for our study will be obtained based on a capacity assessment by the health care provider and will be recorded with a signature. Where applicable, informed assent will be obtained, along with consent from a parent/guardian. Since this is a longitudinal study for five years, the subjects have the option of withdrawing at any time in the study. It is mentioned in the consent forms. Copies of the consent form will go to the subject, the Health Records chart and one copy will be kept by the physicians. A log will also be maintained to characterize the eligible subjects whose parents did not consent to the study.

Involvement in this study will not preclude any subject from receiving a therapy felt to be indicated by the subjects’ responsible physician.
The target number of participants identified and eligible will be 100 obese youth over a 4 year period at each site: SickKids and CHEO. Follow-up will occur for at least 1 year (up to 4 years) in those with OSA treated with PAP.

4.3 Study Population

Inclusion criteria:
1) Age 8 to 17 years. Typically, patients younger than 8 years of age will undergo an adenotonsillectomy as treatment for OSA. Obese kids 8 years or older with OSA usually require PAP therapy;
2) Obesity, defined as a BMI greater than or equal to the 95th percentile for sex and age;
3) Scheduled to undergo a clinically indicated baseline PSG to assess for OSA;
4) Child and parent/guardian are fluent in English.

Exclusion Criteria:
1) Craniofacial abnormalities;
2) Central nervous system lesions;
3) Neuromuscular, neurological, or genetic syndromes;
4) Congenital heart disease and/or diagnosed ventricular dysfunction;
5) Chronic respiratory disease with the exception of asthma;
6) Use of pharmacological sleep aids;
7) Use of medication to treat glucose, hypertension or psychiatric disorders e.g. depression, anxiety;
8) Known co-morbidities like hypertension, fatty liver, dyslipidemia.

4.4 Study Design

4.4.1 Description of Study Design

Visit 1/Sleep Clinic Visit

1. A full explanation of the procedures including informed consent.
2. Physical examination and medical history as per routine clinical care.
3. Pulmonary Function Tests (PFTs) will be conducted as per standard clinical care.
4. Anthropometric measurements, including height, weight, hip, neck, mid-upper-arm circumference, and waist circumference will be taken.

Following baseline visit

1. All subjects will be asked to wear a pedometer as a means to monitor their physical activity level. Pedometers and instructions for its use will be mailed via FedEx to the subjects approximately 7 days prior to the baseline PSG. The research assistant will call the patients when the pedometers are sent out to instruct the patients on their use, and an instruction page will be sent with the pedometer to ensure there are no difficulties. Subjects will return the pedometer to sleep technician on the night of their PSG.
2. Subjects will undergo a clinically indicated overnight PSG.
3. While the sleep technicians are setting up the PSG equipment, parents and subjects will complete the following questionnaires:
a. Sleep Questionnaires – The Pediatric Sleep Questionnaire (PSQ) and Pittsburgh Sleep Quality Index (PSQI) are validated questionnaires that will be used to ascertain a full sleep history. The PSQ will be completed by the parent, and the PSQI will be completed by the subject.

b. Measures of Neurobehavioural Function – The 1) Child Behaviour Checklist, 2) Behavioral Rating Inventory of Executive Function and 3) Connor’s Parent Rating Scale will be used to measure neurocognitive and behavioural function. These will be completed by the parent.

c. Quality of Life Questionnaires – The PedsQL and OSA-18 questionnaires are validated tools to assess health-related quality of life. Both the parent and the subject will complete the PedsQL questionnaire. The OSA-18 questionnaire will be completed by the parent.

d. Physical Activity Questionnaires – The Physical Activity Questionnaire is a validated and accurate assessment of physical activity. This will be completed by the subject.

e. Tanner Staging Questionnaires – The Tanner Staging Questionnaire will assess physical development. This will be completed by the subject.

f. Questionnaire that assesses parental risk factors (i.e. smoking, family history of obesity and OSA) in order to assess whether these factors increase the risk for childhood OSA. This will be completed by the parent.

These questionnaires will take approximately 30 to 45 minutes for the parent, and 15-20 minutes for the subject.

4. During the morning following the PSG, subjects will complete fasting blood work for research purposes for:
   - High sensitivity-CRP (hs-CRP), full blood count (includes CBC & Differential), fasting glucose, fasting insulin, liver enzymes (includes AST, ALP, ALT, gamma GT), fasting lipid profile (includes triglycerides – HDL and LDL cholesterol), hemoglobin (HbA1c), vitamin D, and electrolytes. This will require approximately 16 mL of blood.
   - DNA and inflammatory markers

**Following the PSG**

The baseline PSG will be scored and interpreted as per standard of clinical care. The PSG will categorize subjects into no, mild and moderate to severe OSA. Based on the baseline PSG, subjects will be diagnosed as the following:

- **No OSA** - no further treatment/follow-up will be provided to the subjects. This group will be used as a control group for further analyses.
- **Mild OSA** - subjects will be reviewed in clinic and treatment options discussed according to standard clinical care. Typically, conservative management and/or nasal steroid therapy or leukotriene receptor antagonist will be offered.
- **Moderate-severe OSA** - subjects will be seen in clinic and will be prescribed PAP as per standard clinical care. These subjects will be reviewed on a yearly basis. Yearly follow up assessments include:
a) PAP data will be downloaded and they will be asked to undergo a repeat 
PSG to assess efficacy of PAP treatment for OSA.
b) All the questionnaires administered during the baseline PSG visit will be 
completed again at yearly PSG visits.
c) The subjects will also undergo fasting blood work at each of these visits as 
a follow-up of the cardiovascular and metabolic biomarkers.
d) Additionally, the pedometer will be mailed via FedEx to the subjects 
approximately 7 days prior to each follow-up PSG. Subjects will return the 
pedometer to the sleep technician on the night of their PSG.

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<th>Visit 1- Sleep Clinic</th>
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4.5 Detailed Methodology

4.5.1 Anthropometric measurements
Hip circumference, mid upper arm circumference, height and weight will be collected according 
to WHO standards by the research assistant [21, 22]. Waist measurements will also be collected 
according to NIH standards [23]. BMI will be calculated based on the height and weight 
measurements [24]. Neck circumference will be measured according to published protocol [25]. 
These measurements will be collected during the first clinic visit, and the morning following the 
baseline and follow-up PSGs.

4.5.2 Demographics and medical history
Demographics and a medical history of co-morbidities will be collected in the form of a chart 
review.

4.5.3 Pulmonary Function Tests (PFTs)
PFTs will be performed as per standard clinical care in the PFT laboratory at SickKids. PFTs are 
clinically indicated for all obese children who are seen in sleep/respirology clinic. This is 
because there is a reported high prevalence of asthma and the presence of OSA may increase the 
risk of asthma. Further, skin prick testing is undertaken to evaluate for allergic rhinitis, a
modifiable risk factor for OSA testing for allergies. These clinical data will be recorded to evaluate for interactions between OSA and asthma.

4.5.4 Polysomnography

An overnight PSG will be performed in accordance with the standards of the American Thoracic Society to assess for OSA [26]. A pressure transducer, thermistor, chest and abdominal inductance plethysmography, end-tidal and transcutaneous CO₂ monitoring, pulse oximetry, a snore microphone, 2 leg channels, a chin electromyogram, electroencephalogram, an electrocardiogram, and simultaneous video monitoring will be used as per the American Thoracic Society standards. The presence of snoring will be ascertained by review of the snore microphone recording and observation of the sleep technologist. Signs of upper airway resistance, including observed paradoxical respirations, increased work of breathing and arousal index will also be evaluated. Sleep study parameters will be analyzed for the obstructive apnea-hypopnea index (OAHI) using the American Academy of Sleep Medicine scoring rules for paediatric studies. An apnea event is defined as a reduction in the amplitude of the thermal sensor by 90% or more during two breaths as determined by the baseline breathing pattern, with concomitant continuous or increased inspiratory effort in the period of cessation of airflow. An event will be defined as hypopnea when the duration is more than 2 breaths, the amplitude reduction in the nasal pressure is 30% or more, and the associated oxygen desaturation is 3% or more, or there is presence of arousal or awakening. The OAHI will be calculated as the number of obstructive apneas, hypopneas and mixed apneas divided by the total sleep time in hours. OSA severity will be graded according to accepted criteria. An OAHI of < 1.5 is normal, an OAHI of >1.5 to <5 indicates mild OSA, an OAHI of >5 to <10 indicates moderate OSA, and an OAHI of ≥10 indicates severe OSA [27]. If a subject is diagnosed with moderate-severe OSA, he or she will be prescribed PAP therapy.

4.5.5 PAP Therapy

**PAP Initiation:** If a subject is diagnosed with moderate-severe OSA, PAP initiation and titration with simultaneous PSG (standard practice at each centre) will be performed using standardised equipment and standardised protocols utilised in the Sleep Laboratory at each centre. **PAP Adherence:** Subjects will be followed up on the phone regularly as per standard clinical care and will be reviewed in the PAP adherence clinic twice in 6 months. Standardised questionnaires will address adherence, side effects of PAP usage and symptoms of OSA, as is usual clinical practice. Objective adherence will be obtained at every clinic visit by downloading usage data from the PAP device (Smart cards). **Definition of PAP Adherence:** There are no current defined criteria in children for either successful or unsuccessful PAP adherence. Current adult criteria [28] accords > 4 hours/night of PAP usage as adherence and one paediatric study used 3 hours of PAP usage as adherence [29]. This is problematic in children, as they typically sleep more than adults. Currently, in our institution, PAP usage more than 50% of total sleep time is considered PAP adherence. However, PAP usage data will be analysed in both a dichotomous fashion and as a continuous variable. **Unsuccessful use or non-adherence to PAP:** This will be defined as subjects who wear PAP for 3 days or less per week. Non-adherent subjects will continue to be followed as they still have OSA and are at increased risk for adverse consequences related to OSA.
PAP expertise: This will be provided by a respiratory therapist (RT), and the 3 sleep physicians, as per standard of clinical care.

4.5.6 Pedometers

The Omron HJ-720ITCCAN pocket pedometer is an objective means of assessing free-living physical activity in individuals.

- This device is typically used during the day when a subject is awake and is not currently used to distinguish between wake/sleep cycles and as such, a subject will only be requested to wear it while awake.
- The device can be removed when the individual is sleeping for comfort reasons and when the individual is exposed to water (bathing or swimming) since it is not waterproof.
- As supplemental information to the activity monitor, individuals will keep a diary to record the time of day when the pedometer is worn. This information provides an account of possible missing data in the pedometer output thus providing a valid approach to standardize physical activity data.
- A pedometer will be used to assess physical activity level based on number of steps/day. This will be compared to recommended values for this population [30].
- Each individual will be asked to place the device onto their waist by hooking it onto their pants to hold the monitor in place.
- This will be worn for 7 days prior to the baseline PSG and again 7 days prior to each follow-up PSG.

4.5.7 Fasting blood work

Fasting levels of glucose and insulin will be measured, in order to calculate HOMA-IR, a measure of insulin sensitivity [31]. HOMA-IR values will be converted to z-scores in order to standardize them for sex and age, relative to an overweight/obese reference population [32].

High sensitivity CRP will also be measured as a marker of inflammation. Other metabolic markers that have been associated with OSA and obesity, some of which are now routinely measured as part of the clinical work up in obesity clinics will be collected. These include full blood count (including CBC and Differential), lipid profile including triglycerides (TG), total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol [33] and hepatic enzymes (ALT, AST, ALP, GGT) [34], hemoglobin (HbA1c), vitamin D, and electrolytes, all of which will be measured by standard enzymatic methods. These bloods will be stored and analysed either at SickKids or CHEO. OSA is associated with non-alcoholic fatty liver disease (NAFLD). There is an increased prevalence of OSA in children and adults with NAFLD, independent of BMI [35, 36]. The degree of liver disease has been associated with OSA severity [36, 37]. Positive airway pressure used to treat adults with OSA, improves biochemical (ALT) and radiologic (ultrasound) evidence of NAFLD [38]. There are no pediatric data on the effect of positive airway pressure on NAFLD.

Patient blood results will be shared with his or her clinic sleep physician. If the sleep physician is concerned with any of the results, he or she will provide treatment as per standard clinical care.
4.5.8 Genetic and Inflammatory Markers Testing for Inflammatory Markers at Center for Advanced Research in Sleep Medicine (CARSM)

Participants be asked whether they would like to take part in a genetics sub-study, where analysis for genetics and inflammatory biomarkers will be conducted at the Center of Advanced Research in Sleep Medicine (CARSM). If the participants agree, an additional 25 mL will be collected at the time of fasting bloodwork. An amount of 25 mL is required for thorough blood analysis and to obtain sufficient results. The collection of 25 mL will only be done once, following the first sleep study. Thus, a total of 41 mL of blood will be collected if participants agree to the genetics sub-study. If the participant does not agree to the sub-study, the additional 25 mL of blood will not be collected, thus only the amount of blood that is necessary for fasting bloodwork will be collected (approx. 16 mL).

OSA involves several genetic factors that may contribute to the phenotype [16]. In anticipation of certain new genetic developments in this field, we would like to extract DNA from the blood sample for genetic analysis. This may help in identifying those subjects at the greatest risk for OSA and the complications of OSA, leading to early interventional strategies. As such, the CSCN has linked with the Center for Advanced Research in Sleep Medicine (CARSM) (Hôpital du Sacré-Cœur de Montréal, University of Montreal) that will be linked with a Canadian Adult OSA Database (also under the Umbrella of the CSCN) and the proposed Canadian Obese Children and Youth Database. The blood samples collected following the first sleep study only will be shipped to the CARSM, which will only be used for the purposes of the CSCN study, and will not be shared with institutions or researchers that are not involved with CSCN. Prior to shipment to CARSM in Montreal, blood samples will be temporarily stored in Dr. Theo Morae’s lab at SickKids.

DNA, which contains the genetic code as well as inflammatory markers will be extracted from the sample. Analysis of biological samples may lead to new biological knowledge about sleep, sleep disorders or other disorders linked to sleep alterations (psychiatric, neurological), which in the future may result in new or improved diagnosis and therapies to treat these disorders. Specifically, the DNA sequences will be observed collectively for a human population with sleep problems in order to identify genes that are common among the population. As such, upon completion of the blood analysis, any incidental or unexpected findings will not be communicated to patients.

To protect the identity and privacy, the samples will be de-identified and labelled with a unique study number or ‘code’ before they are sent to CARSM and no other personal identifiers will be sent. The samples will only be linked to anonymous study data. The code linking any personal identifiers to the sample will be kept in a secure and confidential location at SickKids only accessible by the research staff and the Research Ethics Board (REB). All information collected is strictly confidential within the limits prescribed by law, and will only be identified by a code. The blood samples are kept in a locked, secure location and access to this location is restricted. Any remaining blood following the analysis will be destroyed as per CARSM policies.

4.5.9 Questionnaires
The following questionnaires will be administered by the research assistant prior to the baseline sleep study. If the child is diagnosed with moderate-severe OSA, these questionnaires will again be completed by the parents and child at each annual follow-up sleep study visit.

4.5.9.1 Sleep Questionnaires

The Paediatric Sleep Questionnaire (PSQ)

The use of a validated sleep questionnaire will be to ascertain a full sleep history, particularly symptoms. Specifically, the pediatric sleep questionnaire contains 22 symptom items that ask about snoring frequency, loud snoring, observed apneas, difficulty breathing during sleep, daytime sleepiness, inattentive or hyperactive behavior, and other pediatric OSA features, each previously shown to correlate with PSG confirmed OSA in referred children. Responses are "yes" = 1, "no" = 0, and "don't know" = missing. The mean response on non missing items is the score, which can vary from 0 to 1. Previous data suggest that a cutoff value of 0.33 would be most effective in identifying pediatric OSA [39]. Subscales include a 4-item sleepiness scale, a 4-item snoring scale, and a 6-item inattention/hyperactivity scale.

Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index (PSQI) effectively measures the quality and patterns of sleep. It is a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval. Nineteen individual items generate seven “component” scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The sum of scores for these seven components yields one global score. The PSQI can be used for both an initial assessment and ongoing comparative measurements across the health care continuum [40].

4.5.9.2 Measures of Neurobehavioural Function

OSA is associated with cognitive, behavioural and functional deficits in young children. However, there are limited data in older children (>12 years) on the neurobehavioral outcomes of untreated OSA. This is important as the adaptive challenges faced by older children will differ when compared with younger children. The functional neurobehavioral impact of OSA in these children is likely to be unique. This data can be provided by use of specific questionnaires addressing neurobehavioral function. We will utilize three short parent completed questionnaires. These include the Child Behaviour Checklist (CBCL) and the Connor’s Parent Rating Scale which assesses behavioral problems and inattention [41-43]. Specifically the CBCL is a survey of behavioral competencies that use standardized, age-adjusted scores and internalizing and externalizing total behavior difficulties. The Conners’s scale will allow us to assess inattention, distractibility and overactivity. The third questionnaire is the Behavior Rating Inventory of Executive Function (BRIEF) questionnaire which is designed to evaluate executive functioning and memory in a broad range of children [44].

4.5.9.3 Quality of Life Questionnaires
The surveys used to assess health-related quality of life (HRQOL) will incorporate well-validated instruments that are sensitive to developmental changes, have been tested in large healthy populations and are age appropriate. Specifically, we will use: PedsQL™ 4.0 Generic Core Scales - to measure generic HRQOL [45].

4.5.9.4 The OSA-18 Quality of Life Questionnaire
The OSA-18 is an 18 item questionnaire that is used to collect information about 5 subscales that are considered to be elements in quality of life, specific to OSA. Sleep disturbance, physical symptoms, emotional symptoms, daytime function and caregiver concerns. On the basis of the information provided, a summary scale is calculated that ranges from 18 to 126. A value at or above 60 is considered abnormal, indicative of a significant negative impact on quality of life. This validated tool will be an essential component of assessment of disease-specific quality of life for this study [46].

4.5.9.5 Physical Activity Questionnaire
The physical activity questionnaire is a validated and accurate self-report assessment of physical activity in overweight and obese kids. Subjects will complete the physical activity questionnaire that has been validated in children and youth to obtain a general estimation of weekend and weekday activity levels [47]. Elementary school children in grades 4-8 (approximately aged 8-14 years) who are currently in the school system and have recess as a regular part of their school week will complete the Physical Activity Questionnaire for Older Children (PAQ-C)[48]. High school students in grades 9-12 (approximately aged 14-17) who are currently in the school system will complete the Physical Activity Questionnaire for Adolescents (PAQ-A) [48].

4.5.9.6 Tanner Staging Questionnaire
Sleep is crucial for regulating reproductive hormone secretion [49]. As such, sleep fragmentation and/or nocturnal hypoxia secondary to OSA is associated with low sex steroid levels [50-55], which can improve after using PAP. Thus OSA may diminish reproductive hormone secretion during childhood, potentially interfering with normal pubertal maturation [53, 55, 56]. In order to further investigate this, the Tanner Staging questionnaire will be use to assess pubic hair and testes in males and breast and ovaries development in females [57, 58]. The questionnaire provides a pictorial scale of physical development for males and females.

4.5.9.7 Questionnaire for parental risk factors
This questionnaire consists of parental risk factors (i.e. smoking, family history of obesity and OSA) in order to assess whether these factors increase the risk for childhood OSA.

5. Statistical Considerations

Sample Size Calculation:
In addition to the ratio of neck circumference to waist circumference, additional predictors to be considered include BMI z-score, sex, age group (8-12 years, 12-16 years, and 16-18 years), and reported history of snoring, for a total of 6 predictor degrees of freedom. Based on the commonly-used guideline of at least 10 events per predictor degree of freedom, considering OSA as the event of interest, a minimum of 60 cases of OSA would therefore be required. Based on our previous work the prevalence of OSA in this population is estimated to be approximately
36% [59]. A total of at least 180 participants would therefore be required. Based on these estimates, as well as feasibility considerations, we will enroll a total of 200 participants (100 at both the collaborating hospitals), of whom we anticipate 72 to have OSA.

Our secondary analysis concerns cardio-metabolic co morbidity, as measured by HOMA-IR z-score (relative to age- and sex-specific references values from an overweight/obese reference population [32]. The analysis will focus on detecting a difference between treatment-adherent and non-adherent participants in mean change in HOMA-IR z-score, 2 years after initiation of therapy. Preliminary data (n=20) from our CIHR-funded cohort study of obese youth with OSA treated with PAP therapy shows non-significant but potentially clinically important differences in change in HOMA-IR between adherent and non-adherent groups after 6 months. Early estimates from this study show a median increase in HOMA-IR in the adherent group of 0.04 and in the non-adherent group of 4.4. While these estimates have not been standardized for age and sex, the reference values in Shashaji indicate that the median change observed in the non-adherent group would correspond to a change in HOMA-IR percentile of 40% compared to virtually no change in median of the adherent group [32]. We are therefore targeting a large effect size (0.8) for the difference in mean change in HOMA-IR z-score at 2 years. With a sample size of 72 participants with OSA, of whom we anticipate approximately 70% will be adherent to PAP treatment based on our previous work, we will have 80% power to detect this change in HOMA-IR z-score.

**Statistical Analyses:**
The primary analysis will consist of developing and evaluating a prediction model for OSA based on the following predictors: ratio of neck circumference to waist circumference, BMI z-score, sex, age group (8-12 years, 12-16 years, and 16-18 years), and reported history of snoring. Logistic regression modeling will be used to fit the model. Odds ratios with 95% confidence intervals will be tabulated. Here and throughout, two-sided p-values less than 0.05 will be considered statistically significant. The Hosmer-Lemeshow statistic will be used to assess goodness of fit. A receiver operating characteristic curve (ROC) will be produced and the area under the ROC curve will be used to evaluate the predictive performance of the model.

The secondary analysis will evaluate the difference in mean change in HOMA-IR z-score at 2 years between the adherent and non-adherent study participants with OSA using an analysis of covariance for HOMA-IR z-score at 2 years with baseline HOMA-IR z-score as a covariate and group (adherent or non-adherent) as a factor. The statistical significance of the group effect will be evaluated and a 95% confidence interval will be produced. Additional secondary analyses will compare adherent and non-adherent groups for change in lipid profile, liver enzymes, vitamin D, C-reactive protein, and changes in questionnaire scores.

6. Potential Difficulties and Harms

**FOR PARTICIPANTS:**

**Discomfort:** Venipuncture may result in some local discomfort, but will be mitigated by application of topical analgesic/anesthetic cream (EMLA) prior to this procedure. Additionally, participants may experience difficulty initiating and/or maintaining sleep in an unfamiliar environment during polysomnography. This is, however, a clinically indicated test which will be
conducted according to usual protocols, designed to minimize disruption to sleep. There is no pain or discomfort associated with this test.

**Follow-Up Visits:** Annual visits to the hospital will be required for ongoing follow-up. During these clinic visits blood will be drawn for testing and questionnaires will be completed. The clinic visit and blood testing will, however, be relevant for clinical care of this subject population and where possible, will be coordinated with other scheduled hospital visits. The completion of questionnaires can be time-consuming (estimate 30 minutes), but can be done in the clinic waiting room or while waiting for blood tests, minimizing additional time required for each visit.

**Privacy:** There is a very small risk of inadvertent breach of privacy. This is unlikely to occur, as each study participant will be assigned a Study Identification Number. Only this ID number will be used on study materials. No other identifying information will appear on the study material. A single master list of study ID numbers and subject names will be kept within locked offices at each site or electronically on a secure drive that is password protected. The study data will be entered into the REDCap database. The data produced from this study will be stored on a password-protected computer on a secure network in a locked office. Electronic data will be stored on a secure, encrypted online database. Only members of the research team and members of the Research Ethics team will have access to the data. Following completion of the research study the data will be kept for 7 years. The data will then be irreversibly anonymized. The personal health information (PHI) collected for this study will not be used for future projects without prior approval of the Research Ethics Board. It will be published in such a way that identification of subjects whose personal health information is being researched is not identifiable.

**ADDITIONAL CONSIDERATIONS**

**Recruitment of participants:** Recruitment at each site will be enhanced through existing collaboration with each institution’s obesity clinic, which will assist with identification of eligible individuals for this study. Polysomnography is part of the required testing for individuals referred from the community for assessment in obesity clinic and as such is a clinically indicated test, in addition to a research outcome. Individuals with obesity currently awaiting polysomnography will be identified from our wait lists and if eligible, will be offered participation in this study.

**Retention of Participants:** Regular contact from the Respirology healthcare team, by telephone and in clinic, is part of clinical practice for individuals identified to have sleep-disordered breathing and will assist in retention of study participants. This contact will also encourage adherence with PAP therapy, which is known to be challenging in this population [60]. In order to aid with retention of participants, an annual stipend will be provided at each annual study visit and parking expenses will be reimbursed.

**Phlebotomy sample volume:** Given that a moderate volume of blood (50mls) will need to be drawn in order to complete all of the study investigations, it is possible that not all tests will be able to be completed on all study participants. As most study blood tests can be analyzed with a small blood volume, these will be completed on all individuals. Where total blood volume in
excess of amounts allowable would be required, blood collection for analysis of biomarkers will be forgone.

**Prevention of Bias:** Given the small numbers of Pediatric Sleep Medicine physicians in Canada, by necessity, the Principal and Site Investigators will, for the most part, also be the treating physicians of children who come to the sleep laboratory. All investigators will, however, be blinded to the results of study outcomes other than polysomnography results, PAP usage, results of HOMA-IR, liver function tests, lipid profile, CRP and hemoglobin A1C. This is necessary to ensure that results of clinical significance are recognized and addressed. Efforts will be made to ensure that the technicians and statistician involved with study measurements are blinded to other study results. Anonymized data on study outcomes will be sent for centralized analysis in order to minimize bias.

7. Potential Benefits

**To the individual:**
As a detailed evaluation of sleep, neurobehavioral function and metabolic parameters will be conducted on each participant, there may be early detection of medical co-morbidities of SDB, for which timely access to appropriate care will be made available. Through early identification of medical issues, intervention may occur prior to the onset of irreversible end-organ damage, minimizing or preventing impact on future health.

Additionally, regular contact with the study team is a factor associated with increased adherence to PAP therapy, which will be facilitated through ongoing study participation and annual visits.

**To society:**
OSA associated with obesity is a growing problem in the pediatric population. As resources for sleep evaluation are limited, with very lengthy wait lists for PSG[10], identification of clinical predictors of those at highest risk of OSA, such as ratio of neck to waist circumference, [19] will assist with prioritization of children and youth for PSG evaluation.

Furthermore, OSA is associated with multi-organ system co-morbidities and substantial (> 200%) increased healthcare utilization, which decreases when OSA is treated [61, 62]. Identification and treatment of OSA therefore will decrease the burden of care for individuals affected and health care resources for society. Finally, through early identification of OSA and its co-morbidities, this study provides an opportunity for early intervention, before irreversible end-organ damage has occurred, which may mitigate future co-morbidity and healthcare burden.

8. References

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