Title: **Cardiometabolic risk and obesity in adolescents with Down syndrome**

Short Title: Cardio risk factors in DS youth – Aim 1

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ABBREVIATIONS AND DEFINITIONS OF TERMS

AAP	American Academy of Pediatrics
ABAS-II	Adaptive behavior assessment system-Second edition parent form
AE	Adverse events
AH	Abdominal height
BES	Body Esteem Scale for Children
BF	Body fat
BG	Blood glucose
BP	Blood pressure
CEBQ	Child eating behavior questionnaire
CHOP	The Children's Hospital of Philadelphia
CMR	Cardiometabolic risk
CNMC	Children's National Medical Center
CPP	Central pulse pressure
CRP	C-reactive protein
CTRC	Clinical and Translational Research Center
DS	Down syndrome
DXA	Dual energy x-ray absorptiometry
EMR	Electronic medical record
FRS	Stunkard Figure Rating Scale
HbA1c	Hemoglobin A1c
HDL-C	High-density lipoprotein cholesterol
HFI	Home food inventory
HOMA-IR	Homeostasis model assessment – insulin resistance
HRQOL	Health-related quality of life
Hs-CRP	High-sensitivity C-reactive protein
IL-6	Interleukin-6
ISI	Insulin sensitivity index
IWQOL-Kids	Impact of weight on quality of life-Kids
LDL-C	Low-density lipoprotein cholesterol
LV	Left ventricular
MCV	Mean corpuscular volume
NMR	Nuclear magnetic resonance
OGTT	Oral glucose tolerance test

PAI 1	Plasminogen Activator Inhibitor
PDAY	Pathological Determinants of Atherosclerosis in Youth
PedsQL	Pediatric Quality of Life Inventory
PeRC	Pediatric Research Consortium
PHI	Protected Health Information
PWA	Pulse wave analysis
PWV	Pulse wave velocity
QOL	Quality of life
RCT	Randomized controlled trial
REE	Resting energy expenditure
REC	Research Enhancement Core
SD	Standard deviation
T1DM	Type 1 diabetes
TC	Total cholesterol
TG	Triglycerides
VLDL-C	Very low-density lipoprotein cholesterol
WC	Waist circumference
WHR	Waist hip ratio

ABSTRACT

Context:

Down syndrome affects 1 per 800 births and is one of the most common causes of developmental disability in the US. Life expectancy for Down syndrome has increased significantly: estimated median survival in the US in 1997 was 49 years. Down syndrome is associated with an increased risk for obesity, with an estimated prevalence of 47-48% in adults and 30-50% in children with Down syndrome. Adolescents with Down syndrome are more likely to have increased adiposity compared to unaffected peers and may be at increased risk for obesity-related co-morbidities, such as type 2 diabetes and cardiovascular disease. How one defines obesity in DS is not clear. Individuals with DS have short stature and possibly increased adiposity, and the body mass index (BMI) used to define obesity for otherwise healthy populations may not accurately depict body fatness or capture cardiometabolic risk in DS.

Objectives:

Primary: To compare the relationship between BMI-Z score and cardiometabolic risk (CMR) factors in youth with DS, and in age-, sex-, race-, ethnicity- and BMI-Z score-matched controls.

Secondary: To examine the relationship between obesity, lifestyle (physical activity, nutrition), body image, and quality of life in DS, and to explore barriers to maintaining a healthy weight in children with DS.

Study Design:

Observational cohort study

Setting/Participants:

The Children's Hospital of Philadelphia Clinical and Translational Research Center and Children's National Medical Center.

A total of 260 evaluable male and female subjects ages 10-20 years of any race or ethnic background will be recruited: 155 subjects with DS (with and without significant CHD) and 105 non-DS controls, matched for age, sex, race, ethnicity, and BMI-Z. Approximately 600 subjects will need to be screened in order to achieve this number of evaluable subjects.

Study Measures:

Anthropometrics (height, weight, BMI), DXA derived measures (lean body mass, fat mass), blood glucose, plasma insulin, oral glucose tolerance tests (glucose tolerance, insulin sensitivity index, insulin resistance (HOMA-IR)), HbA1c, lipids, non-HDL cholesterol, lipoprotein subclass analysis, hs-CRP, IL-6, PAI 1, , adiponectin, leptin, TSH & T4, blood

pressure, pulse wave velocity, pulse wave analysis, left ventricular mass, quality of life, physical activity, body image, nutrition.

PROTOCOL SYNOPSIS

Study Title	Cardiometabolic risk and obesity in adolescents with Down syndrome
Funder	National Institutes of Health (NIH)
Study Rationale	DS is associated with an increased risk for obesity. Adolescents with DS are more likely to have increased adiposity compared to unaffected peers and may be at increased risk for obesity-related co-morbidities, such as type 2 diabetes (T2DM) and cardiovascular disease (CVD). Congenital heart disease (CHD) affects approximately 50% of individuals with DS; the NHLBI Working Group on Obesity and Other Cardio-vascular Risk Factors in Congenital Heart Disease highlighted the high prevalence of obesity in the setting of CHD, and called for studies to identify obesity measures that are more sensitive than BMI as well as studies of CVD risk prevention**.** Unfortunately, clinicians caring for obese adolescents with DS with or without CHD have little scientific evidence upon which to base guidance regarding cardiometabolic risk (CMR): data regarding CVD risk and prevalence of pre-diabetes and T2DM in obese adolescents with DS are lacking. The measure of body fatness which best predicts CMR in DS is not known. We plan to compare BMI and other measures of body fatness in healthy controls and adolescents with DS to determine which measures best capture CVD and/or T2DM risk. These data will equip medical providers with the tools to better assess risk, initiate prevention measures, and guide screening in adolescents with DS.
Study Objective(s) **Part 1:**	**Primary** **To compare the relationship between BMI-Z score and cardiometabolic risk factors in youth with DS, and in age-, sex-, race-, ethnicity-, and BMI-Z score-matched controls.** a) Compare cardiometabolic risk factors [non-HDL cholesterol, lipoprotein subclass particles, blood pressure, insulin resistance (measured by HOMA-IR), insulin sensitivity index (ISI), glucose tolerance, inflammatory markers, and adipokines)] in adolescents with DS and in matched controls. b) Determine the extent to which adiposity (fat mass by DXA) explains this relationship in DS vs. controls. c) Assess cardiac end organ injury (pulse wave velocity (PWV) and left ventricular mass) in relationship to BMI-Z score in adolescents with DS and controls. Determine whether BMI-Z or other measures of adiposity (skin-fold thickness, waist circumference) better discriminates adolescents with DS at increased cardiometabolic risk. **Hypothesis:** DS is associated with worse cardiometabolic risk factors for a given BMI-Z compared to controls. This difference arises at least in part, from increased adiposity.

	Secondary **To examine the relationship between obesity, lifestyle (physical activity, nutrition), body image, and quality of life in DS, and to explore barriers to maintaining a healthy weight in children with DS**. a) Compare the relationships between BMI-Z, and body image and quality of life in adolescents with DS with those of matched controls. b) Identify DS-specific barriers (i.e., nutrition, behavioral issues, body image, feeding behaviors, parental-and self-imposed activity restrictions) to maintaining a healthy lifestyle. **Hypothesis:** Obesity negatively impacts body image and quality of life in adolescents with DS. Obesity is associated with less physical activity. Psychological, behavioral, physical conditions unique to DS impede maintenance of healthy lifestyle and weight.
Study Design	This is cross sectional study of two groups of adolescents: healthy controls and subjects with DS.
Subject Population key criteria for Inclusion and Exclusion:	**Inclusion Criteria** Control Group 1. Males and females age 10 – 20 years 2. Parental/guardian permission (informed consent) and if appropriate, child assent Down Syndrome Group 1. Male and females age 10 – 20 years 2. Diagnosis of Down syndrome 3. Parental/guardian permission (informed consent) and if appropriate, child assent **Exclusion Criteria (Both Groups)** 1. Major organ system illness (such as leukemia, for DS), except for T2DM 2. Cyanotic congenital heart disease and/or pulmonary hypertension (as described by most recent echo report in subjects with CHD) 3. Medically unstable congenital heart disease 4. Pregnancy 5. Genetic syndrome known to affect glucose tolerance 6. Familial hypercholesterolemia 7. Currently treated with medications known to affect insulin sensitivity other than diabetes agents in known T2DM subjects (e.g. oral glucocorticoids or high-dose inhaled steroids(>1000

	mcg/day) in the last month) or lipid profiles (statins, high dose vitamin A), or atypical antipsychotics
Number Of Subjects	Total Number of Subjects Overall and at CHOP: Total n = 260. 155 with DS and 105 controls. At CHOP: Total n = 210 (130 with DS and 80 controls). Total Number of Study Sites: Two – The Children's Hospital of Philadelphia and Children's National Medical Center
Study Duration	Each subject's participation will last approximately 7-8 hours for those with BMI ≥ 85%ile for age and sex (undergoing OGTT), and 5-6 hours for those with BMI <85%ile; physical activity will be determined over 7 days in the home setting; Three twenty-four hour dietary recall interviews of parents and children will be conducted research bionutritionists by telephone.
Study Phases	**Screening & Recruitment:** Potential participants (DS group and control group) will be identified through the ambulatory electronic medical record (EMR) and primary care clinics. DS subjects may also be recruited from Endocrinology, Trisomy 21 and Cardiology clinics, DS community events, the DS Growing Up Study and local advertisements. Control subjects may also be identified through local advertisement. After potential subjects are identified, parents/guardians will be contacted to screen for eligibility. Eligible, willing subjects will be scheduled for a morning study visit after a 12 hour overnight fast. **Phase 1:** Parental/guardian permission and child assent (if applicable) will be obtained prior to any study related procedures being performed. Subjects will undergo the following: physical exam/pubertal assessment, anthropometric measurements, vital sign assessment, fasting blood draw, 2-hour oral glucose tolerance test (OGTT), Pulse wave velocity (PWV) and Pulse Wave Analysis (PWA), echocardiography. Subjects and their parent/guardian will complete the following questionnaires: The Pediatric QOL inventory (PedsQL™), Stunkard Figure Rating Scale (FRS), Body Esteem Scale for Children (BES), Adolescent Sedentary Activity Questionnaire (ASAQ), Child Eating Behavior Questionnaire (CEBQ), Child Feeding Questionnaire (CFQ), Adaptive Behavior Assessment System-Second Edition (ABAS-II) Parent Form, the Home Food Inventory (HFI), and family history of cardiometabolic conditions. Dietary history will be obtained by three twenty-four hour dietary recall interviews will be performed over the telephone. Physical activity will be assessed by accelerometry using SenseWear® Armbands. Armbands are worn for 7 consecutive days.
Efficacy	N/A in Aim 1

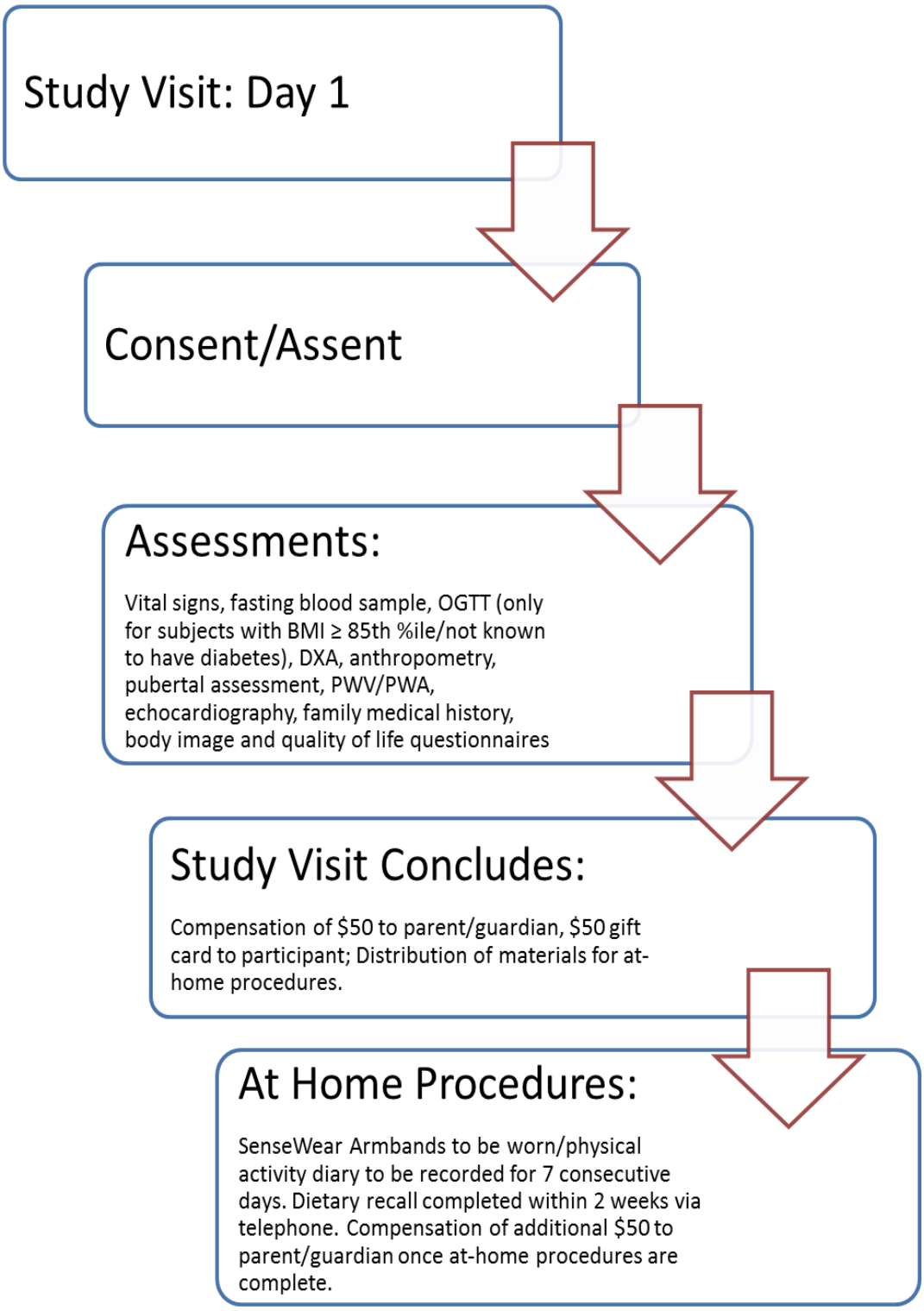
Evaluations	
Pharmacokinetic Evaluations	N/A
Safety Evaluations	Adverse events are not anticipated. Any AEs and SAEs will be reported to the CTRC and the IRB within the allotted timeframe.
Statistical And Analytic Plan	. Data Analysis: Descriptive data analysis will summarize distributions of all risk factor measures Subjects with DS will be compared to age-, sex-, race-, and BMI-Z matched controls, first by comparison of risk factor distributions by DS status. Next, separate regression models will be fit for each risk factor, with DS as the exposure of interest, adjusted for potential confounders.
DATA AND SAFETY MONITORING PLAN	The study team will be responsible for data management and collection and are responsible for the accuracy and completeness. Only investigators and study team members that have completed appropriate IRB training/approval are eligible to collect and work on information collected from this study. Data will be entered into REDCap. Using REDCap checking tools, the data manager will confirm completeness and valid values weekly and inform any errors or omissions to the investigative team for prompt resolution. Original data will be recorded directly onto Case Report Forms (CRF) by the study team. All CRFs will be locked in a file cabinet in a locked office. In order to guard against disclosure of protected health information, each study participant will be assigned a unique 4 digit identification code. The code will be kept in a locked cabinet in the PI and/or study coordinator's office.

TABLE 1: SCHEDULE OF STUDY PROCEDURES

Study Phase	**Screening**		
Visit Number		**1**	
Study Days		**1**	
Verbal Consent	X		Will be done prior to screening
Review Inclusion/Exclusion Criteria	X		
HFI	X		Will be done at home, following screening but before study visit
Informed Consent/Assent		X	
Demographics/Medical History		X	
Physical Examination		X	
Vital Signs: BP, HR, RR		X	
Height and Weight		X	
Pregnancy Test (If applicable)		X	
Fasting Blood Glucose & Insulin Levels		X	
Fasting Lipid Panel: TG, TC, HDL-C, LDL-C		X	
Lipoprotein subclass analysis		X	
OGTT (only in subjects with BMI≥85%ile, not known to have diabetes)		X	
HbA1c		X	
Hs-CRP		X	
PAI 1		X	
Adiponectin (total and HMW)& Leptin		X	
TSH & T4		X	
PWV & PWA		X	
DXA		X	
Echocardiography		X	
Pubertal status		X	
Smoking status		X	
Family history of CVD, dyslipidemia, T2DM		X	
Physical activity		X	Worn for 7 days after study visit
Body image questionnaires:(PedsQL, IWQOL-Kids, IWQOL-Parents, CEBQ, CFQ, ABAS-II, FRS, ASAQ, BES, Body satisfaction scales, Physical activity/armband diary)		X	Physical activity/armband diary will be completed at home after study visit. All other questionnaires will be completed during the study visit.
Dietary Intake (24 hour dietary			X X X

recall done 3 times)			

FIGURE 1: STUDY DIAGRAM- Sample (exact procedure order may vary)



1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Understanding the role of obesity and body composition in cardiometabolic (CMR) factors in Down syndrome (DS). DS affects 1 per 800 births[12] and is one of the most common causes of developmental disability in the U.S. Life expectancy for DS has increased significantly; estimated median survival in the US was 49 years in 1997[13]. DS is associated with an increased risk for obesity[14], with an estimated prevalence of 47-48%[15] in adults and 30-50%[16] in children with DS. Concurrent with the physiologic increase in insulin resistance that accompanies puberty, adolescents with DS are more likely to have increased adiposity compared to unaffected peers, and may be at increased risk for obesity-related comorbidities, such as type 2 diabetes (T2DM)[17] and cardiovascular disease (CVD). Moreover, congenital heart disease (CHD) affects approximately 50% of individuals with DS[5]; the NHLBI Working Group on Obesity and Other Cardio-vascular Risk Factors in Congenital Heart Disease highlighted the high prevalence of obesity in the setting of CHD, and called for studies to identify obesity measures that are more sensitive than BMI as well as studies of CVD risk prevention.[6] Unfortunately, clinicians caring for obese adolescents with DS with or without CHD have little scientific evidence upon which to base guidance regarding CMR: data regarding CVD risk and prevalence of pre-diabetes and T2DM in obese adolescents with DS are lacking.

In 1977, DS was proposed to be an atheroma-free model; atheroma was completely absent at autopsy in five institutionalized adults with DS, while atheroma was present in institutionalized adults without DS[18]. Individuals with DS were hypothesized to be protected from atherosclerotic disease. Subsequent studies both supported[19], [20], and contradicted[21], [22] this conclusion. Group differences in lipid profiles were not found in the original study, but others identified a more atherosclerotic lipid profile in DS [23-26]. Recently, after excluding deaths caused by CHD, three large cohort studies reported increased mortality due to CVD in DS [27-29]. Hill et al[28] found a standardized mortality ratio of 6.2 for cardiovascular deaths, with a ratio of 3.9 for ischemic heart disease specifically. Mortality due to diabetes mellitus is also increased, with a standardized mortality ratio of 11.4 [28], however T1DM and T2DM were not differentiated. These data suggest increased CVD risk in DS, but the relative contributions of obesity versus factor(s) intrinsic to DS have yet to be delineated. Increased life expectancy in DS demands that we better understand obesity, characterize CMR, and identify the best screening measures for this risk, with the goal of minimizing health disparities for this at-risk population. This study will examine the association of obesity with CMR in DS during adolescence, a critical period marked by pubertal insulin resistance and transition to potential independence. Our study has direct implications for daily medical care of individuals with DS. The marker which best predicts CMR in DS is not known. We plan to evaluate which measure of adiposity best captures CVD and/or T2DM risk in DS to equip medical providers with the tools to better assess risk, initiate prevention measures, and guide screening in adolescents with DS.

Understanding the relationship of obesity to body image, QOL, and physical activity, and explore barriers to healthy weight in adolescents with DS. Childhood obesity may contribute to significant psychosocial complications[30]. In general, obese children report poorer QOL.[31] Children and adults with DS have low levels of physical activity [32, 33]. Children with CHD also

tend to be less physically active than peers[34, 35], and self-concept may limit engagement in physical activity; [36] thus, a cycle of poor self-esteem may feed physical inactivity and obesity. Among children with DS, factors such as competing family responsibilities, reduced skills; and paucity of accessible programs likely contribute to low physical activity[37]. Body image, QOL, and physical activity in relation to BMI have not been explored in adolescents with DS. Cognitive and physical complications of DS, as well as parental perceptions, can make traditional community-based weight loss programs difficult to access and utilize effectively. Identification of barriers to obesity prevention and management is crucial if effective programs are to be developed. This study will explore body image, QOL, physical activity patterns, eating behaviors, and parental perception of activity risk and tolerance in adolescents with DS, both with and without CHD.

Thus, we will characterize the relationship between obesity and CMR in DS, fill a critical knowledge gap regarding CVD and T2DM risk, and clarify screening measures for these risks. We will also explore psychosocial implications of increased weight and barriers to healthy weight in adolescents with DS, to establish the foundation for future studies addressing these important aspects of obesity prevention and treatment.

1.2 Compliance Statement

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia and Children's National Medical Center Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with The Children's Hospital of Philadelphia and Children's National Medical Center IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

This study will examine the association of BMI and body composition as measured by DXA with CMR in adolescents with DS.

2.1 Primary Aim 1

The primary objective is to compare the relationship between BMI-Z score and cardiometabolic risk factors in youth with DS, and in age-, sex-, race-, ethnicity- and BMI-Z score- matched controls.

a) Compare cardiometabolic risk factors [non-HDL cholesterol, lipoprotein subclass particles, blood pressure, insulin resistance (measured by HOMA-IR), insulin sensitivity index (ISI), glucose tolerance, inflammatory markers, and adipokines)] in adolescents with DS and in matched controls.

b) Determine the extent to which adiposity (fat mass by DXA) explains this relationship in DS vs. controls.

c) Assess cardiac end organ injury (pulse wave velocity (PWV) and left ventricular mass) in relationship to BMI-Z score in adolescents with DS and controls. Determine whether BMI-Z or other measures of adiposity (skin-fold thickness, waist circumference) better discriminates adolescents with DS at increased cardiometabolic risk.

2.2 Secondary Aim

The secondary objective is to examine the relationship between obesity, lifestyle (physical activity, nutrition), body image, and quality of life in DS, and to explore barriers to maintaining a healthy weight in children with DS.

a) Compare the relationships between BMI-Z, and body image and quality of life in adolescents with DS with those of matched controls.

b) Identify DS-specific barriers (i.e., nutrition, behavioral issues, body image, feeding behaviors, parental and self-imposed activity restrictions) to maintaining a healthy lifestyle.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This observational cross-sectional study will involve one study visit for all groups. All participants will be consented before any procedures. During the study visit both groups will receive: urine pregnancy test (for female subjects), fasting blood sample, OGTT (if BMI≥85% percentile), DXA scan, anthropometric measurements, pubertal status exam, PWV and PWA, echocardiography, family history of CVD/dyslipidemia/T2DM, QOL, body image, eating behaviors and other questionnaires. Three twenty-four hour dietary recall interviews of parents and children will be conducted by research bionutritionists by telephone, using the multi-pass method. Accelerometers will be distributed for home use.

3.2 Total Number of Study Sites/Total Number of Subjects Projected

3.2.1 Duration of Study Participation

The study duration for both the DS and control group will be approximately 5-8 hours for the 1-day visit. The dietary intake phone interview will be approximately 30 minutes each time, totaling approximately 1.5 hours. SenseWear armbands will be worn in the home environment for seven consecutive days.

3.2.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at 2 investigative sites in the United States: The CTRC at CHOP and the CSR at CNMC.

Recruitment will stop when approximately 260 evaluable subjects are enrolled (155 DS subjects and 105 control subjects). It is expected that CHOP will enroll 130 subjects and CNMC will enroll 25 subjects into the Down syndrome group, and that CHOP will enroll 80 subjects and CNMC will enroll 25 subjects into the control group. Approximately 600 subjects

overall (450 at CHOP and 150 at CNMC) will be screened, or "enrolled," to produce 260 evaluable subjects.

3.3 Study Population

3.3.1 Inclusion Criteria

The study plans to recruit approximately equal numbers of females and males, and is open to participants regardless of their race or ethnic background. We will not restrict enrollment based on gender, race or ethnicity, and will actively recruit members of minority groups. We anticipate that the sample will be predominantly Caucasian and African American with smaller numbers of Hispanic and Asian minority groups since these are not well-represented in the CHOP and CNMC catchment areas.. We anticipate recruiting a number of participants who self-identify as mixed race. Very few families of American Indian/Alaska Native and Native Hawaiian or Other Pacific Islander groups reside in the Philadelphia area and are not likely to be represented in this study.

In the event that a non-English speaking participant wishes to enroll in the study, we will use the Hospital Interpreter Services to locate an interpreter that can assist the study and the family to achieve this goal.

3.3.2 Inclusion Criteria

DS Group

1) Males and females age 10 to 20 years.

2) Diagnosis of DS

3) Parental/guardian permission (informed consent) and if appropriate, child assent

Control Group

1) Males and females age 10 to 20 years
2) Parental/guardian permission (informed consent) and if appropriate, child assent

3.3.3 Exclusion Criteria

Both DS and Control Group

1) Major organ system illness (such as leukemia, for DS) except T2DM

2) Cyanotic congenital heart disease pulmonary hypertension (as described by last echo report in subjects with CHD), or congenital heart disease considered medically unstable by the study cardiologists

3) Pregnancy

4) Genetic syndrome known to affect glucose tolerance

5) Familial hypercholesterolemia

6) Currently treated with medications known to affect insulin sensitivity other than diabetes agents in known T2DM subjects (e.g. oral glucocorticoids or high-dose inhaled steroids (>1000mcg/day) in the last month) or lipid profiles (statins, high dose vitamin A), or atypical antipsychotics

Subjects that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

4 STUDY PROCEDURES

4.1 Study Visit

- Informed Consent: 30 minutes/as long as needed for parent/guardian/participant to fully comprehend the study involvement
- Fasting blood draw: 15 minutes
- Pubertal Status Exam: 5 minutes
- OGTT (Only done in subjects with BMI ≥85%ile, not known to have diabetes): 2 hours
- Vital Signs: 10 minutes
- DXA scan: 10 minutes
- Anthropometric measurements: 15 minutes
- PWV and PWA: 30 minutes
- Echocardiography: 45 minutes
- Family history of CVD, dyslipidemia, T2DM: 10 minutes
- QOL, body image, and other questionnaires: 1 hour

4.1.1 Visit 1

- Fasting Blood Sample: Will be completed by nursing staff. A total of approximately 12.5 mL (approximately 2½ teaspoons) of blood will be obtained from all participants. Subjects who do not undergo OGTT will be allowed to eat after the fasting blood sample.

- OGTT: A 2 hour OGTT will be done only in subjects with BMI≥85%tile, not known to have diabetes. A total of approximately 7.5 mL (1½ teaspoons) of blood will be obtained. The OGTT will be performed by nursing staff. A blood drawing IV will be placed. Baseline BG and insulin will be obtained. Subjects will ingest glucose solution (1.75 g/kg; maximum of 75 g) over 2 minutes. BG and insulin will be draw at 30, 60, 90, and 120 minutes via a blood drawing IV. For subjects refusing a blood drawing IV, BG and insulin will be drawn fasting and at 120 minutes. If a subject refuses the OGTT, only fasting labs will be obtained. Subjects who undergo OGTT will be allowed to eat after the OGTT is completed.

- Anthropometric Measurements: Performed by trained research anthropometrists, in order to assess body composition.

- DXA Scan: The scan measures body composition and will be conducted and analyzed by trained staff.
- Pubertal Status: Tanner staging of puberty will be performed by a pediatric endocrinologist team member. This exam will take place in a private exam room.

- Pulse Wave Velocity (PWV) and Pulse Wave Analysis (PWA): The PWV and PWA will be performed by a trained technician[123]

- Echocardiography: Left ventricular mass will be measured by a trained sonographer and analyzed by the study cardiologist.

- Family History of CVD, Dyslipidemia, T2DM: The study coordinator or appropriate member of the investigative team will administer a CRF to collect relevant patient medical history, family history of CVD/dyslipidemia/T2DM, sedentary and physical activity for each participant. Each question will be verbally communicated to the parent/participant, and the team member will show the questions as they are read, as appropriate.

- Questionnaire Assessment: The following questionnaires (PedsQL, IWQOL, BES, FRS, ASAQ, CEBQ, CFQ, ABAS-II, Body Satisfaction Scales) will be administered by the study coordinator or appropriate member of the investigative team. Each questionnaire will be verbally communicated to the parent/participant, and the team member will show the questions as they are read, as appropriate. The HFI and physical activity/armband diary will be self-administered at home.

    - PedsQL - The Pediatric Quality of Life Inventory uses a modular approach to measuring health related QOL in healthy children, adolescents, and those with acute and chronic health conditions. Scales include: 1) physical functioning, 2) emotional functioning, 3) social functioning, and 4) school functioning, which yield summary scores of a total scale score, a psychosocial health summary score, and the physical health summary score.
    - IWQOL-Kids - The Impact of Weight on Quality of Life – Kids (IWQOL-Kids) © is a validated self-report measure of weight-related quality of life for youth ages 11-19. This condition-specific QOL assessment is a 27 item questionnaire that yields a total score and 4 domain scores, including physical comfort, body esteem, social life, and family relations. It has been used in children, adolescents, and in special populations.
        - IWQOL-Kids – Parent Form - The Impact of Weight on Quality of Life Kids – Parent Form (IWQOL-Parents) is a measure of the parent perception of weight-related quality of life. This condition-specific QOL assessment is a 27 item questionnaire that yields a total score and 4 domain scores, including physical comfort, body esteem, social life, and family relations.
    - BES – the Body Esteem Scale for Children is a 20-item, yes/no questionnaire designed to assess children's attitudes and feelings about their body and appearance.

- FRS – The Stunkard Figure Rating Scale depicts 9 male and 9 female figures, ranging in size from very thin to very overweight. It is used to assess perceptions of current and ideal body size.
- ASAQ - The Adolescent Sedentary Activity Questionnaire is a reliable assessment of a comprehensive range of sedentary behaviors that occur in school-aged young people. In this measure, the parent will estimate the amount of time spent engaging in various sedentary activities during each day of a typical school week and weekend.
- CEBQ – The Child Eating Behavior Questionnaire[157] is a reliable and valid, 35 item questionnaire measuring appetite and eating style (satiety responsiveness, food enjoyment, food responsiveness, slowness in eating, food fussiness, desire to drink, emotional over-eating and emotional under-eating).
- CFQ – The Child Feeding Questionnaire measures parental feeding practices, assessing parental beliefs, attitudes, and practices regarding child feeding. It is designed for parents of children in the age range of 2-11 years of age.
- ABAS-II – The Adaptive Behavior Assessment System-Second Edition is widely used to evaluate individuals with intellectual and developmental disabilities measuring daily living skills (what people actually do, or can do, without the assistance of others). It assesses adaptive behavior in individuals 5-21 years of age.
- Body Satisfaction Scales – The body satisfaction scales measure the level of satisfaction with body size, shape and weight.
- HFI – The Home Food Inventory is a valid assessment of the home food environment. Families will be mailed the HFI prior to the study appointment and will be asked to bring the completed survey to the study appointment.
- Physical activity/armband diary – Participants will be asked to keep a record of the times that they did not wear the physical activity armband and of their activities on the days that they wore the armband. The diary packet will be sent home with each participant, along with a postage-paid envelope to return the diary.

- SenseWear® Armbands (Body Media, Inc.). Participants will be asked to wear armband accelerometers for 7 days, 24 hours per day, for 1 week with the exception of when they are bathing/showering or if participating in water activities, such as swimming. Information collected will include amount and intensity of physical activities, sedentary activities, and sleep. There are no direct benefits to participants of this assessment, but the data gleaned from these assessments will provide valuable information about the lifestyle habits of children and adolescents with Down syndrome.

4.2 Follow-up Phase

Dietary Intake Assessment: The twenty-four hour dietary recall will be completed by the research bionutritionists in the two to three weeks following the study visit. Three phone calls

(to gather information on two weekdays, one weekday) will be averaged to complete a single report.

4.3 Subject Completion/Withdrawal

Subjects may withdraw from the study at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the investigators for lack of adherence to study treatment or visit schedules, AEs, and/or pregnancy. The investigators may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject completes the clinical study. If the Investigators become aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents and on the CRF.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening and Monitoring Evaluations and Measurements

5.1.1 Physical Examination

Evaluations obtained during the study visit include Tanner staging of puberty by a pediatric endocrinologist.

5.1.2 Vital Signs

Vital signs will be recorded while the subject is sitting down by the nursing staff. BP will be measured by auscultation three times, after a five minute rest in a quiet area with the subject in a seated position; the average of the 2nd and 3rd measurements will be used.

5.1.3 Laboratory Evaluations

A fasting blood sample will be performed for the following laboratory evaluations. For those individuals undergoing the OGTT, this will be done with insertion of the intravenous (IV) line before glucose has been ingested. For individuals not requiring an OGTT, the fasting blood samples will be obtained by a routine needle stick. When necessary, commercial kits for the individual lab tests will be purchased by the investigators. Blood sampling will be performed for the following laboratory evaluations.

1. Lipid panel (TG, TC, HDL-C, LDL-C)

2. Lipoprotein subclass analysis

3. Blood glucose (BG)

4. Plasma insulin

5. High sensitivity C-reactive protein (hs-CRP)

6. Interleukin 6 (IL-6)

7. Plasminogen Activator Inhibitor 1 (PAI 1)

8. Adiponectin (total and HMW) & Leptin

9. TSH and T4

10. HbA1c

5.1.4 Glucose Tolerance Test

Glucose Tolerance (2-hour oral glucose tolerance test (OGTT)) will be performed by nursing staff. Subjects will be told to eat a high carbohydrate diet for 3 days before the study. Topical anesthetic ointment at the phlebotomy sites will be offered the morning of the visit. Baseline BG and insulin will be obtained. Subjects will ingest glucose solution (1.75 g/kg; maximum of 75 g) over 2 minutes. BG and insulin will be drawn at 30, 60, 90, and 120 min via a blood-drawing IV. However, the investigators realize that not all subjects will tolerate placement of the blood drawing IV, particularly given that children with Down syndrome may have developmental delays. For these subjects, BG and insulin will be drawn fasting, subjects will ingest glucose solution as described above, and a second blood draw will occur at 120 minutes. OGTT will be interpreted based on American Diabetes Association criteria[98]: Fasting BG: <100 mg/dl= normal, 100-125mg/dl = impaired fasting glucose (IFG), ≥126 mg/dl = diabetes; 2-hour BG <140 mg/dL = normal, 140-199 mg/dL = impaired glucose tolerance (IGT), ≥200 mg/dL = diabetes.

5.1.5 Pulse Wave Velocity (PWV) and Pulse Wave Analysis (PWA)

Pulse Wave Velocity (PWV) and Pulse Wave Analysis (PWA) will be performed in the by a trained professional. . Aortic PWV measurements are performed in the supine position after at least 5 minutes rest using the right carotid and right femoral arteries, with a Sphygmocor PVx System (AtCor Medical, West Ryde, Australia) device. PWA will provide novel, supplemental data about central pulse pressure (CPP) in our subjects. The same software and equipment as for PWV are used, on the right radial artery. CPP is measured using radial artery tonometry.[99]

5.1.6 Anthropometry

Weight measured by digital electronic scale (Scaletronix) and stature on a stadiometer (Holtain). Age- and sex-specific Z-scores generated based on CDC 2000 growth charts[100] so that DS and non-DS groups will be compared by the same reference. Body proportions and fat distribution measurements (sitting height, arm,waist and hip circumference, and skin fold thickness at triceps, biceps, subscapular, and suprailiac sites) will be measured.

5.1.7 Left Ventricular (LV) Mass

LV mass will be measured by echocardiography, as described by the American Society of Echocardiography's guidelines[101]. Per these guidelines the equation of Devereux et al will be used[102]. LV Mass Index (LV mass/height2.7), has been used to normalize LV mass to body size[103]. However, this index increases with decreasing height, which is not ideal for children with DS. Use of centile curves and z-scores[104] is another strategy to account for

lean mass (LM), which may also be different in children with DS. We will measure LM by DXA and can thus normalize LV mass directly.

5.1.7.1Pregnancy Testing

A urine pregnancy test will be performed for female subjects. A positive pregnancy result will be disclosed to the participant only. Pregnancy results will be disclosed to the parent/guardian if the subject gives the investigators permission. Subjects found to be pregnant during the visit will not be able to enroll in this study. The investigators will counsel the subject and guide them to seek the appropriate care.

5.1.8 Safety Evaluation

The study investigators will be responsible for safety monitoring. A routine needle stick or an IV line used for drawing blood may cause some discomfort (topical anesthetic cream will be available), bruising, and rarely fainting. Use of the anesthetic cream may cause an allergic reaction. Although high adiposity is possible in participants with BMI<85%ile and might contribute to abnormal glucose tolerance, we will limit the subjects undergoing OGTT 1) to minimize participant burden in this cohort of children with developmental disabilities, and 2) on the likelihood of finding cardiometabolic abnormalities. We confined our IR assessment to HOMA-IR rather than IVGTT or clamp studies--considered the gold standard for IR assessment-- in the spirit of sensitivity to these children with intellectual disabilities and their families. We will obtain interim time points within the OGTT to calculate ISI, a measure of whole body insulin sensitivity. This requires a minimum of 5 time points, necessitating the placement of a blood-drawing IV. Given the children's developmental disabilities, some children may not tolerate this procedure—in this subset only time points 0 and 120 will be drawn and ISI omitted from their analyses.

Participants will be fasting 12 hours prior to their study visit. This may cause hunger pangs, upset stomach, headache, or light-headedness. If the participant shows any signs of clinical instability or definitively decides to discontinue participation, the study visit will be ended. Possible side effects of the OGTT include temporary high or low blood sugar. A risk of the OGTT includes an upset stomach. Participants will be monitored by a nurse or doctor during the study procedures. Episodes of low blood glucose will be treated with oral or IV glucose when indicated.

During the study visit, if a participant is found to have any previously unknown medical conditions, they will be referred to their pediatrician for care.

This protocol also involves exposure to radiation from the DXA scans. However, this involves a very small radiation exposure, which is unlikely to cause any untoward effects to the participant. Being that the protocol may be unsafe to an unborn child, pregnancy is an exclusion criterion of the study.

The noninvasive measures of arterial stiffness and central pulse pressure are done via Pulse Wave Velocity and Pulse Wave Analysis. This poses little risk to the patient.

The patient will be notified of any clinically relevant abnormal test result(s) performed by CLIA certified labs, by mail and or by phone. Because of batching of samples, there may be a

significant time delay between the study visit and result notification. It will be recommended that results, especially abnormal results, be discussed by the guardian with the participant's primary care physician. In the case of study referrals by the primary care provider, the investigators may send study results via email, phone, and/or mail to the primary care physician directly. Studies performed by non CLIA labs will not be disclosed.

Any adverse events will be reported as per the study site policies.

6 STATISTICAL CONSIDERATIONS

6.1 Data Analysis

We will screen approximately 200 children with DS to enroll 155 evaluable children with DS. Approximately 50% will have CHD, which will be hemodynamically significant in approximately 50-75%. Subjects with minor, hemodynamically insignificant lesions (such as a small PDA) will be grouped with subjects without CHD.

Primary Aim: The primary aim is to compare the relationship between BMI-Z and cardiometabolic risk factors in youth with DS and matched controls. This relationship may be different in children with DS with and without CHD. In order to model the analyses appropriately, the primary outcome variables will be compared between children with DS with and without hemodynamically significant CHD. If the groups differ, subsequent analyses will include a dummy variable indicating presence/absence of hemodynamically significant CHD. Descriptive data analysis will summarize distributions of all risk factor measures (age, weight, BMI-Z, non-HDL-C, lipid levels, lipoprotein subclass particles, BP, HOMA-IR, ISI, inflammatory markers, adipokines, CPP, PWV, LV mass, gender, race, ethnicity, pubertal stage, pre-diabetes and diabetes status, physical activity). Subjects with DS will be compared to age-, sex-, race-, and BMI-Z matched controls, first by comparison of risk factor distributions by DS status. Next, we will fit separate regression models for each risk factor, with DS as the exposure of interest, adjusted for potential confounders, including pubertal stage, family history of CVD, dietary fat intake, and smoking history. Interaction terms among confounders will be used when data frequencies permit. Initial regressions will confirm the size and sign of associations of subject characteristics and CVD risk factors. Complementary analyses will implement propensity scores, using logistic regression to model DS as a function of observed covariates, and then will compare DS and non-DS children for each endpoint using weighted analyses. Weights will be calculated by the inverse of the fitted probability of DS[106]. This method will standardize the comparisons of CVD risk factors by differences in observed characteristics of DS and non- DS children and thus will permit statements such as ―If a sample of children otherwise comparable were alternatively DS and non-DS, what would be the differences in CVD risk factors? Finally, we will perform sensitivity analyses as to the potential impact on our results from unobserved potential confounders. Initial analyses will determine whether children with DS differ from their non-DS counterparts in terms of CVD risk factors and will become the basis for addressing the questions in the four sub-aims.

6.1.1 Aim 1a) BMI-Z scores and DS – CMR factors

To analyze this aim, the statistical model noted above will be modified to include an interaction term between DS and BMI-Z score for each CVD risk factor. An important interaction term will reflect varying association of BMI-Z by disease status. Based on preliminary data we estimate 50% of subjects with DS will have BMI≥85%ile and undergo an OGTT. We will compare prevalence of IGT or IFG and T2DM between groups. (Although DS is associated with increased risk for T1DM, this would most likely present acutely and have already been diagnosed if present.)

6.1.2 Aim 1b) DXA-measured FM This sub aim will make use of the model in Aim 1a. Inclusion of FM in explaining CMR factors in the model should offset any statistical interaction between BMI-Z and DS status.
Aim 1c) PWV [and LV Mass] Using the model outlined previously, we will add PWV as an indicator of end organ damage to assess additional information provided by PWV, especially with regard to the interaction of BMI-Z and CVD risk factor levels. This approach will be used for CPP and LV mass as well.

6.1.3 Aim 1d) Discrimination ability of BMI-Z and other screening measures This analysis will be confined to children with DS, and will require for each CVD risk factor the determination before analysis of a threshold of high versus low, in order to evaluate discrimination. With BMI-Z, and each risk factor taken individually, we shall estimate the c-statistic, a measure of discrimination representing the area under the ROC curve of the tradeoff of sensitivity and specificity, for each screening measure as a predictor of high vs. low risk. Finally, we shall compare the additional discriminatory power of adding one screening measure to an existing screening measure, such as WC to BMI-Z to determine whether an additional screen is worth the expense and time. These contrasts will be implemented using the methods outlined by Pepe and colleagues[122].

6.1.4 Secondary Aim

Initial data analysis will be descriptive to examine distributions of QOL, body image and other measures in this AIM. Models for QOL and body image, with DS and BMI-Z as the exposure of interest, adjusted for potential confounders (e.g., sex, ethnicity) will be linear regressions and alternatively ordinal regressions depending on the measure distributions. The latter regression model assumes only ordered rather than continuous dependent variables. Estimates of interest will be the main effects (association of BMI-Z on QOL and body image), and the interaction of DS and these factors (does the presence of DS modify associations of interest?).

Stata software (version 11.1 or later; Stata Corp., College Station, Texas), supplemented with SAS v 9.2 (SAS Institute, Cary NC) and R (The R Foundation for Statistical Computing, Vienna, Austria) will be used for statistical analysis. Methods outlined by Pepe and colleagues are now implemented in Stata v 11.

6.2 Sample Size and Statistical Power

Primary Aim: Assuming 155 evaluable children with DS and 105 evaluable children without DS for analysis and conservatively using nonparametric methods for comparison of CVD risk factors, power exceeds 0.85 to detect a between-group difference of 0.35 standard deviation (SD). For example, non-HDL-C is our primary CMR outcome measure, and 0.35 SD

corresponds to a difference of 7 mg/dl. A difference of 10 mg/dl is considered clinically relevant by pediatric cardiologists.

Secondary Aim: Power for the paired design can be assessed by assuming paired comparisons of residuals of a regression of BMI-Z on QOL and body image measures. Assuming that the outcomes, and thus residuals, will be continuous, power = 0.86 to detect as significant as little as 0.25 SD in differences between DS children and controls using nonparametric tests (Wilcoxon) that would suitable for both continuous and ordered data. Thus, the sample should have substantial power to demonstrate even small differences between DS and matched controls.

6.3 Potential Problems/Feasibility

If we find no differences in CMR factors between youth with DS and controls, the resulting estimates (and confidence bounds) will still generate crucial information, given the literature claims of decreased CVD risk in DS, and scarcity of data on T2DM risk. Confidence bounds on estimates will allow us to rule out clinically important differences. We might find that DS children are at decreased CVD risk compared to unaffected controls. Such information would help guide clinicians caring for children with DS, and prevent unnecessary screening. Although high adiposity is possible in participants with BMI<85%ile and might contribute to abnormal glucose tolerance, we will limit the subjects undergoing OGTT 1) to minimize participant burden in this cohort of children with developmental disabilities, and 2) on the likelihood of finding cardiometabolic abnormalities. We confined our IR assessment to HOMA-IR rather than IVGTT or clamp studies--considered the gold standard for IR assessment-- in the spirit of sensitivity to these children with intellectual disabilities and their families. We will obtain interim time points within the OGTT to calculate ISI, a measure of whole body insulin sensitivity. This requires a minimum of 5 time points, necessitating the placement of a blood-drawing IV. Given the children's developmental disabilities, some children may not tolerate this procedure—in this subset only time points 0 and 120 will be drawn and ISI omitted from their analyses. Measures of LV mass may still be confounded by mild valve regurgitation or EKG conduction abnormalities. We are aware of this issue, and will interpret de-identified results with the expertise of the study cardiologist(s). They advocate acquiring this data, given the cardiometabolic implications of LV mass. The technique has not been used previously in this population and will generate novel data.

7 SAFETY MANAGEMENT

7.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study.

7.2 Adverse Event Reporting

The Investigator is responsible for recording and reporting unanticipated problems related to research that occur during and after study treatment. The plan for Adverse Event reporting should be consistent with the study siteIRB Guidelines. Since the study procedures are not greater than minimal risk, SAEs are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including

SAEs) these will be reported to the IRB in accordance with the study site policies. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

7.3 Medical Emergencies (if applicable)

The investigators do not anticipate any medical emergencies with this minimal risk study. However, there is a possibility that study participants may have low or high blood glucose or side effects resulting from the OGTT. The study participants will be monitored throughout testing. The study coordinator, a nurse or physician will be present during these procedures. If necessary, episodes of significant hypoglycemia will be treated immediately with oral or IV glucose (although unlikely).

8 STUDY ADMINISTRATION

8.1 Data Collection and Management

Data Management CHOP CTRC Informatics Core will create case report forms (CRF) and REDCap database for data capture. REDCap is an NIH-supported web-based data management software designed by Vanderbilt University investigators. Of special benefit to the proposed investigation is the interface between EPIC (the EMR) and the research database that allows for use of clinical information for research purposes. For data not already incorporated in EPIC, once the investigative team confirms complete and accurate data, it will be entered into REDCap. Using REDCap checking tools, the data manager confirms completeness and valid values weekly and informs any errors or omissions to the investigative team for prompt resolution. Real-time data checking ensures prompt creation of an analysis dataset upon data acquisition completion. Tables generated by REDCap will be exported and merged for use with Stata and SAS statistical packages as needed for immediate access to the research team.

The PI is responsible for the accuracy and completeness of data collection and management. The PI may designate qualified individual(s) to collect data and manage data. Only investigators and research staff that have completed appropriate IRB training and approval and are listed on the IRB approved protocol are eligible to collect and work on information from the study. Future studies that may use patients or data collected from this study must have separate approved IRB protocols and consent forms, if applicable.

Recruitment data will be recorded onto the screening questionnaire after the verbal consent is recorded. Original data will be recorded directly onto CRFs by the study coordinator or a study investigator. Copies of laboratory, physical exam, anthropometric, DXA, PWV, and PWA results will be received through inter-office mailing, picked up directly, and sometimes through email. This information also will be recorded onto CRFs while the originals may be kept at the testing site.CRFs will be kept in a locked filing cabinet in a locked room at all times. All information will be transferred to REDCap, a secure, web-based application supported by the CHOP Research Institute. The password to log onto the database will be unique to each member of the study team. CNMC study staff will be assigned to a REDcap user group that will restrict their access so they may view and edit solely the information of subjects enrolled at CNMC. The CNMC Principle Investigator will have access to view/edit data of all subjects.

Note that only limited PHI (date of visit, date of birth) is included in the REDcap database. CHOP will have read-write access for data collected at both sites. CHOP will require access to edit records for both sites because several evaluations will be analyzed at CHOP, and reports including data from both CHOP and CNMC sites will be forwarded to the CHOP team, who will then import or enter the data into REDCap. Written informed consent granting permission for other site(s) to access PHI will be obtained from all subjects.The CRF and REDCap database will be designed by the CTRC Informatics Services.

All data and records generated during this study will be kept confidential in accordance with institutional policies and on HIPPA subject privacy. The investigators/study team members/site personnel will not use such data and records for any purpose other than for conducting the study.

As a way to minimize the chance of PHI (protected health information) from being disclosed, a unique identification code will be used for each participant. The key to this code will be kept in a locked file in the PI/Study Coordinator's office.

If any publications result from this research, the participant will not be identified by name/PHI.

The information collected as part of this study will be kept for 6 years or until the completion of the study (whichever is longer). At that time, the information collected will be destroyed or all identifiable information will be removed. All keys will be destroyed at this time, as well.

8.2 Confidentiality

All data and records generated during this study will be kept confidential in accordance with institutional policies and HIPPA's guidelines on subject privacy. The investigators/study personnel will not use such data and records for any purpose other than conducting the study.

8.3 Regulatory and Ethical Considerations

8.3.1 Data and Safety Monitoring Plan

Study progress and safety will be reviewed quarterly (and for frequently if needed) by the PI. Review of the rate of subject accrual, adherence to inclusion/exclusion criteria will occur quarterly to assure that participants meet the eligibility criteria. There will be ongoing collection of data on adverse events and compliance to the treatment protocol throughout the study by research staff, which will be reviewed monthly by the study team and PI. If there is an incidental finding deemed by the PI to be clinically relevant, the primary care provider will be notified. The parent/guardian will be notified and asked to follow up with the subject's primary care provider if during the visit; a subject is noted to have blood pressure that is $\geq$ the 95^{th} percentile for age, gender, and height. We will notify the parent/guardian/primary care provider with clinically relevant abnormal labs. Specifically, lipid levels/insulin levels/blood glucose levels/OGTT results/TSH and T4. Note that some labs, such as lipids and insulin, are run in batches and reporting of results may be delayed.

8.3.2 Incidental Findings

Participants will have the option to consent for any incidental findings to be shared with their primary care provider. If there is an incidental finding deemed by the PI to be clinically

relevant, the primary care provider will be notified. The parent/guardian will be notified and asked to follow up with the subject's primary care provider if during the visit; a subject is noted to have blood pressure that is ≥ the 95th percentile for age, gender, and height. We will notify the parent/guardian/primary care provider with clinically relevant abnormal labs. Specifically, lipid levels/insulin levels/blood glucose levels/OGTT results/TSH and T4. Note that some labs, such as lipids and insulin, are run in batches and reporting of results may be delayed.

8.3.3 Risk Assessment

The risks of participating in this study are relatively small and the investigators believe the overall study is of minimal risk to the participants. An OGTT will only be administered to subjects that have BMIs ≥ 85th percentile, not known to have diabetes.

Expected risks to the subject are as follows:

- Blood draw may result in temporary discomfort from the needle stick, bruising, fainting, weakness, and rarely an infection at the site
- Possible side effects of the OGTT include temporary high or low blood sugar and upset stomach
- Anthropometric measurements and pubertal status exam poses minimal risks
- Exposure to radiation during DXA scan
- Sharing of private health information (PHI) including dietary intake, demographic information, health history and medical information
- Pulse wave velocity and pulse wave analysis poses minimal risks
- 12 hour overnight fasting may cause hunger pangs, upset stomach, headache, or light-headedness
- Blood pressure measurement by auscultation may cause temporary numbness/tingling in the arm
- SenseWear® Armbands worn for 7 consecutive days may cause skin irritability
- Sensitive psychosocial issues may arise during questionnaire assessment

A small amount of blood (about 3 teaspoons) will be drawn at major assessment points. Adolescents may experience some degree of discomfort, bruising, or lightheadedness with fasting blood draws. There is also a risk of infection when blood is drawn. These occurrences are rare, generally mild, and respond to conservative treatment.

The OGTT is frequently used to screen for diabetes and impairments in glucose regulation in children both within the confines of a study and in routine clinical practice; additional intermediate blood draws will be performed for glucose and insulin in order to assess insulin sensitivity. Because of these intermediate time points for blood collection, an intravenous line will be placed, which can be associated with discomfort and a small risk of infection. The use of EMLA cream is optional to minimize discomfort to the study participant. Experienced pediatric research nurses will perform the OGTT; OGTT are routinely performed in this setting. Seven blood samples are required and the volumes of blood will be minimized to reduce the risk of anemia. The concern with OGTT is hypoglycemia occurring 2-3 hours after consumption of the dextrose load. If hypoglycemic

symptoms develop, blood glucose will be checked. In the event of hypoglycemia (blood glucose<60 mg/dL), the study will be terminated and carbohydrates consumed by the subject. Blood glucose will be monitored until normalization is assured. To minimize subject burden, particularly in this cohort of children with developmental disabilities, we will perform OGTT only in subjects with BMI≥85%. While the aim of the study is to uncover cardiometabolic risk factors in children with DS and to uncover whether adolescents with DS have greater risk for a given BMI z-score, glucose intolerance is unlikely in children with BMI<85% and the yield of doing OGTT in this subgroup is relatively low. Our team felt that it was crucial to be sensitive to the burden of participation placed on these children with intellectual and developmental disabilities and their families.

Participation in the study involves risks associated with the small amount of radiation exposure associated with the DXA whole body scan for body composition assessment; the total radiation EDE from DXA scans is less than 3 μSv (or 1 mrem). This total amount of radiation is less than the exposure to daily background radiation at sea level (3,000 μSv per year) and is therefore considered minimal risk. Females of childbearing potential undergo a urine pregnancy test prior to the DXA scans. Pregnant females do not have DXA scans performed in order to protect the unborn fetus.

The anthropometric assessment involves measurements of height, weight, circumferences and skinfold thickness. There is a very minor possibility of bruising from the skinfold thickness measurements. The exam will be performed by trained anthropometrists experienced in obtaining measurements in children at all levels of cognitive ability. The measurements are obtained in a private room. The parent is permitted to stay with the child if it increases their comfort with the exam.

The puberty assessment will be performed by pediatric endocrinologists in a private setting. The procedure will be explained to the child in advance, and the parent is permitted to be present if preferred by the child. The exam is performed by highly experienced personnel who are familiar with minimizing distress associated with the exam.

SenseWear® Armbands (Body Media, Inc.). There is little to no risk associated with measuring physical activity, sedentary behavior, and sleep with armband accelerometers. These accelerometers fit comfortably on the participant's arm and can easily be removed should he/she become uncomfortable. Some patients may experience skin irritation, particularly when sweating, with the devices in which case they can adjust them or in rare instances, remove them if necessary. To prevent irritation, parents will be instructed to clean the armband with mild soap and water when the child removes it to bathe or shower. If irritation does occur, parents will be instructed to use Aquaphor or Vaseline where the fabric of the band is to treat and prevent further irritation. If a child is allergic to metal they will not be required to complete this assessment.

A licensed clinical psychologist with expertise in working with children and adolescents, will meet with families concerning sensitive psychosocial issues that may arise during assessments or intervention. Any emotional upset will be handled with appropriate support to the participants and their caregivers. Further, if a behavioral health concern arises

during the study needing further evaluation and treatment, the clinical psychologist will assist with a referral.

If a subject shows any signs of clinical instability or definitively decides to discontinue participation, the study visit will end.

8.3.4 Potential Benefits of Study Participation

There may be no direct benefit and benefits will be to future patients, Science and society. Participants may directly benefit from identification of abnormalities such as diabetes from the OGTT. Participants may indirectly benefit from identification of abnormalities such as dyslipidemia and hypothyroidism from the fasting blood draw. If clinically relevant abnormalities are found, the family will be notified. With the consent from the family, clinically relevant tests results will be shared with the subject's primary care physician. Only studies performed by CLIA certified labs will be disclosed. Participants found to have an impaired fasting glucose or impaired glucose tolerance will be referred appropriately for further management and treatment.

The physical activity questionnaires and dietary recall may potentially lead the participants/parents/guardians into awareness about making healthy lifestyle options in diet and exercise.

There is also a potential indirect benefit in helping scientists and health providers further understand the relationship between obesity and Cardiometabolic risk in Down syndrome youth.

8.3.5 Risk-Benefit Assessment

The benefits to participation in this trial outweigh the potential risks. Clinicians caring for obese adolescents with Down syndrome with or without CHD have very little scientific evidence upon which to base guidance regarding Cardiometabolic risk. Data regarding prevalence of pre-diabetes and T2D and on CVD risk in obese adolescents with Down syndrome are lacking. This study will have direct implications for the daily medical care of individuals with Down syndrome. Currently, we do not know whether BMI is the best marker of cardiometabolic risk in Down syndrome. We plan to determine which measure of adiposity best captures CVD and or diabetes risk in Down syndrome, so that primary care providers will know what measure to use to assess risk in adolescents with Down syndrome (i.e. BMI, waist circumference, or skin fold thickness). Medical providers will then be able to use the appropriate measure for prevention, and to guide screening.

8.4 Recruitment Strategy

Potential subjects will be identified using the protocol inclusion and exclusion criteria. Subjects will be recruited from multiple sources, including local primary care, Endocrinology, Cardiology and Trisomy 21 clinics, local advertisements, previous studies (if they gave permission to be contacted), and DS community events. The DS Connect recruitment registry will e-mail registry participants the IRB-approved recruitment flyer.

A study website will also be used as a source of information for potential DS and control subjects.

The Recruitment Enhancement Core (REC) will e-mail or postal mail potential control subjects using the IRB-approved REC letter for control subjects. This letter will contain opt-out language that allows study staff to contact participants via one phone call who have not opted-out 2 weeks following the letter being sent.

After the initial telephone screening, subjects that agree to participate and qualify for the study will be scheduled for an appointment. Verbal consent will be obtained by the study team prior to the screening.

8.5 Informed Consent/Assent and HIPAA Authorization

Prior to conducting the screening interview and prior to mailing the family the HFI questionnaire, verbal consent from the subject (if 18 years or older)/parent/guardian/legal authorized representative will be obtained and documented by the study coordinator/study investigator. A description of the procedures involved in the study, as well as the risks/benefits will be provided verbally as part of this process. Additionally, it will be stressed that any questions are appropriate and that all aspects of the study are voluntary.

Prior to conducting the study visit procedures, the study coordinator will obtain consent and if appropriate assent from the participant. A waiver of minor assent by the study coordinator will be obtained for participants not capable of assenting. The consent/assent process will take place in a private room. It will be stressed again that participation in the study is voluntary and that any questions can and should be raised. Consent and assent will be documented by the parent/guardian/participant's signatures on the approved consent documents. A description of the procedures involved in the study, as well as the risks and benefits, will be provided verbally as part of the consent process.

Consent/assent documents will be maintained in the participant's study file and documented. The parent/guardian will receive a copy of the signed document(s).

8.5.1 Consent for Down syndrome subjects 18 – 20 years of age

Ability to provide consent will be ascertained by the study team. Initially, the team will assess the participants' ability by engaging them in conversation to obtain a brief, general sense of their ability to comprehend and communicate. If ability to provide consent seems likely, the team will then explain the following to confirm that the study participant understands all of the essential elements of the consent form:

a. The purpose of the research study. We will explain that the study is trying to find out which tests and measurements will best capture the risks of heart disease and type 2 diabetes in children and adolescents with DS. We will ask them to explain in their own words what the study is about.

b. Study procedures will be explained with the aid of a picture book that depicts the study visit and at-home procedures. We will then ask them to explain in their own words what each procedure will be like.

c. The staff will clearly explain that the study participant does not have to participate in the study, that they can change their mind about being in the study at any time, and that no one will be disappointed, upset or consider it a failure if they decide they do not want to continue with the study. Subjects will be asked to explain in their own words that they understand that they do not have to participate in the study and that there are no consequences if they choose not to.

d. We will explain that their information will be kept private to the best of our ability. We will explain the risks of each procedure. We will also explain that they may learn more about their health from the study, and that their health may improve from taking the thyroid hormone pills, but that it is also possible that they may not benefit from the study. Participants will be asked to explain in their own words what they understand about the risks and benefits of the study.

If it is deemed by the study team at any of the above points that the subject is unable to consent for themselves, the subject's legally authorized representative/health care representative will provide the written informed consent on the subject's behalf.

8.5.2 Assent Procedures

Assent will be obtained and documented on the consent form for children capable of assenting. DS subjects age 18-20 years of age that are not capable of consenting for themselves will have assent documented in the consent form. If the capability of some of the participants is limited in comprehending the study and that they cannot reasonably be consulted, assent will not be obtained. In these cases, the investigators will document it on the consent form. Investigators will obtain assent whenever possible.

8.5.3 Waiver of Documentation of Consent

Waiver of documentation of consent is being sought for screening procedures, as the subject over age 18/parent/guardian/legal authorized representative will be contacted by telephone and obtaining a signature will not be feasible. As described in (8.5), verbal consent/HIPAA authorization will be obtained and documented by the study coordinator/study investigator prior to conducting the screening interview and prior to mailing the family the HFI.

8.5.4 Waiver of Assent

Waiver of assent is being sought for screening procedures, as subjects may not be present when parents/guardians/legally authorized representatives are contacted

8.6 Payment to Subjects/Families

All study procedures and expenses will not be billed to the participant. The total reimbursement for the study visit is $150. The parent/guardian will receive a total of $100 to offset the burdens of transportation/parking/time off work/babysitting fees/meals. The parent/guardian will receive $50 immediately after the study visit and an additional $50 once all post-visit procedures are complete. The participant will receive a $50 gift card for their efforts toward the study.

In the event that any study procedure cannot be completed on the date of the study visit and the participant returns on another date to complete any pending study procedure(s), the parent/guardian will be reimbursed with $50 to offset the burdens of the time and effort needed to come in for the visit.

9 PUBLICATION

The results of this study may be submitted for consideration for presentations at national meetings and/or publication in academic journals. At no time will any PHI from this study be disclosed for any presentation(s) or journal article(s).

10 REFERENCES

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