

Title: **Levothyroxine treatment and cardiometabolic outcomes in adolescents with DS**

Short Title Aim 2 Levothyroxine and DS

Drug Name(s): Levothyroxine

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Study Principal Investigator

Andrea Kelly, MD, MSCE

11 NW 30 Main Building, Children's Hospital of Philadelphia, 34th St. & Civic Center Blvd.

Philadelphia, PA, 19104

Phone: 215-590-3420

Email: kellya@email.chop.edu

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Abbreviations and Definitions of Terms

AAP	American Academy of Pediatrics
ABAS-II	Adaptive behavior assessment system-Second edition parent form
ANTITIPO	Thyroid peroxidase (microsomal) antibodies
ANTITG	Thyroglobulin antibodies
AE	Adverse events
ASAQ	Adolescent Sedentary Activity Questionnaire
BES	Body esteem scale for children
BG	Blood glucose
BP	Blood pressure
CEBQ	Child eating behavior questionnaire
CFQ	Child feeding 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
Hs-CRP	High-sensitivity C-reactive protein
IL-6	Interleukin-6
IWQOL-Kids	Impact of weight on quality of life-Kids
LDL-C	Low-density lipoprotein cholesterol
LV	Left ventricular
NMR	Nuclear magnetic resonance
OGTT	Oral glucose tolerance test

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
SD	Standard deviation
T1DM	Type 1 diabetes
TC	Total cholesterol
TG	Triglycerides
TSH	Thyroid stimulating hormone
PAI-1	Plasminogen Activator Inhibitor 1
VLDL-C	Very low-density lipoprotein cholesterol
WC	Waist circumference

PROTOCOL SYNOPSIS

Study Title	Levothyroxine treatment and cardiometabolic outcomes in adolescents with Down syndrome
Funder	National Institutes of Health (NIH)
Clinical Phase	N/A
Study Rationale	The American Academy of Pediatrics (AAP) recommends annual screening for thyroid dysfunction in children and adolescents with Down Syndrome (DS) ⁽¹⁾ . Mild elevations in TSH are common in DS. Recently, subclinical hypothyroidism has been associated with increased cardiovascular (CVD) risk in the general adult population, and debate rages as to whether subclinical hypothyroidism should be treated. Moreover, the potential benefits of treating subclinical hypothyroidism in DS are not known. This study will examine the effect of levothyroxine hormone treatment on body composition, cardiometabolic risk (CMR) factors, and quality of life (QOL) in children with DS and subclinical hypothyroidism.
Study Objective(s)	<p>Primary</p> <ul style="list-style-type: none"> To determine the effect of levothyroxine replacement on BMI-Z, fat mass, and CMR factors in pre-adolescents and adolescents with DS and subclinical hypothyroidism. <p>Secondary</p> <ul style="list-style-type: none"> To determine the effect of levothyroxine replacement on QOL, lifestyle (physical activity, nutrition) and body image in pre-adolescents and adolescents with DS and subclinical hypothyroidism.
Test Article(s)	Levothyroxine (starting dose 0.5 – 1 mcg/kg daily)
Study Design	Single blinded randomized controlled trial using switching replication design.
Subject Population	Inclusion Criteria
key criteria for Inclusion and Exclusion:	<ol style="list-style-type: none"> Males and females, ages 8 – 20 years Diagnosis of Down syndrome Subclinical hypothyroidism: TSH level between 5 – 10 mIU/L, normal T4

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4. Parental/guardian permission (informed consent) and if appropriate, child assent
 5. Females who are ≥ 11 years of age or who are menarchal must have a negative urine/serum pregnancy test
 6. Committed to adherence to levothyroxine treatment and study completion

Exclusion Criteria

1. Pregnancy
2. Type 1/Type 2 diabetes
3. Chronic medical conditions or medication use that can affect growth, nutrition, blood glucose, insulin secretion, or thyroid function (such as lithium or certain seizure medications)
4. Current use of levothyroxine or anti-thyroid hormone
5. Cyanotic congenital heart disease, or pulmonary hypertension (as described by last echo report in subjects with CHD), or congenital heart disease considered medically unstable by the study cardiologists

Number Of Subjects	Total number of subjects overall and at CHOP: 29 enrolled for 20 completers overall; 11 enrolled for 6 completers at CHOP Total number of study sites: 2: The Children’s Hospital of Philadelphia and Children’s National Medical Center
Study Duration	Participation will last 18 months All subjects will be observed for 6 months (observation period). Only subjects with persistently elevated TSH range (5-10 mIU/mL) at 6 months will be enrolled into 1 of 2 randomized groups. <u>Group 1</u> : 6 months of placebo followed by 6 months of levothyroxine. OR <u>Group 2</u> : 12 months of levothyroxine.
Study Phases Screening Study Treatment Follow-Up	Screening & Recruitment: Subjects from Aim 1 (IRB# 9233) that qualify and consent to being contacted for future studies can be recruited into Aim 2. For subjects recruited from Aim 1, Aim 1 data will serve as baseline data and a screening visit is not necessary. Additional subjects will be recruited from CHOP’s Pediatric Research Consortium (PeRC), CNMC primary care clinics, DS

community events, other studies, local advertisements, local primary care practices, Trisomy 21 and Cardiology clinics, and Endocrinology clinic, where children with DS are routinely referred for thyroid function abnormalities.

Subjects not recruited from Aim 1 will have an initial interview and a screening visit.

We plan to recruit 29 subjects with the goal of 20 subjects who complete the 18 month study.

Study Treatment: A single blinded randomized trial of levothyroxine treatment (starting dose 0.5 – 1 mcg/kg daily) using a switching replication design. Subjects will be randomized into 2 groups of 10.

(Group 1) Delayed treatment group: 6 months of placebo followed by 6 months of levothyroxine treatment

(Group 2) Immediate treatment group: 12 months of levothyroxine treatment

Follow-Up: Studies will be obtained at baseline, 6 months, 12 months, and 18 months. Interim thyroid studies will be checked at 3 months, 6 weeks after randomization (month 7.5); month 13.5 and 6 weeks after any dose adjustment.

Efficacy Evaluations

TSH

T4

Anthropometry (HT-Z, WT-Z, BMI-Z),

body composition by DXA,

PWV & PWA

Blood lipid panel, non-HDL-C, lipoprotein subclass analysis, glucose, plasma insulin, HOMA-IR, hs-CRP, IL-6, PAI-1, adiponectin, leptin,

QOL, Body Image, physical activity assessment.

Safety Evaluations

To monitor for safety, data will be made available to investigators every 3 months. The PI will not be blinded to the treatment arm, will be making dose adjustments, and for safety purposes, TSH and T4 data will be available to the PI as soon as it is available.

Interview at 4 and 6 weeks after treatment initiation and at each visit to document intensity and frequency of any AE.

Any AEs and SAEs will be reported to the CTRC and the IRB within the allotted time frame.

Statistical And Analytic Plan

Standard mixed effects piecewise longitudinal data model ⁽²⁾ will be used to estimate in a single model both longitudinal (within subject) and cross-sectional (across subject) effects of the intervention. Cross-sectional comparisons will be possible for the second 6-month period of observation when ½ of subjects will be switched to treatment while the other ½ will take a placebo.

DATA AND SAFETY MONITORING PLAN

The Biostatistics and Data Management Core (BDMC) at CHOP and the Investigative team will be responsible for data management. The Investigators will review TSH and T4 levels as they are available and other data every 3 months. A data safety monitoring board (DSMB) will be established. The DSMB will consist of at least four members including a biostatistician, a developmental pediatrician from outside of The Children’s Hospital of Philadelphia, a pediatric endocrinologist, and a senior faculty member with experience in clinical research and in care of children and adolescents with complex chronic illness. The DSMB will choose its chair. Specific rules and protocols for all reviews will be established in a formal protocol, and approved by the DSMB, prior to the first DSMB meeting. The DSMB will work jointly with the trial statisticians and clinical investigators to establish specific criteria to accomplish its tasks. The Project PI will establish procedures for identifying classes of AEs, documenting them on an Adverse Experience Report (AER) form, and then reporting them regularly to the DSMB.

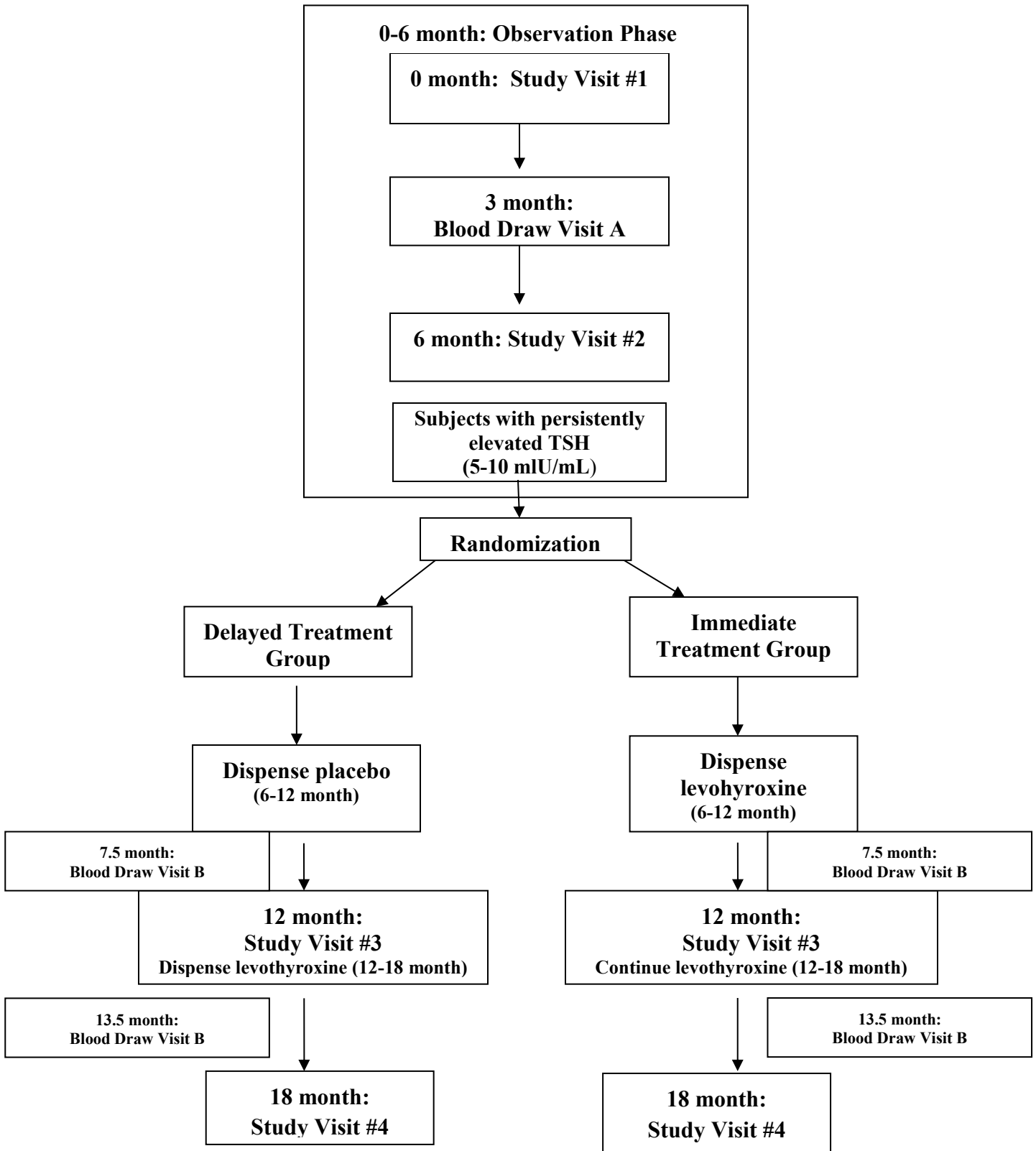
TABLE 1: SCHEDULE OF STUDY PROCEDURES: BOTH GROUPS

Study Phase	Observation			Treatment				
Visit	Study Visit #1 (0 month)	Blood Draw Visit A (3 month)	Study Visit #2 (6 month)	Randomization (no visit)	Blood draw Visit B (7.5 month)	Study Visit #3 (12 month)	Blood Draw Visit C (13.5 month)	Study Visit #4 (18 month)
Study Days	1		1			1		1
Informed Consent/Assent	X (written consent for subjects not recruited from IRB #9233)	X (verbal consent for subjects recruited from IRB #9233)	X (written consent for subjects recruited from IRB #9233)					
Review Inclusion/Exclusion Criteria	X							
Demographics/ Medical History	X		X			X		X
Vital Signs: BP, HR, RR	X		X			X		X
Height and Weight	X		X			X		X
Pregnancy Test	X		X			X		X
Prior/Concomitant Medications	X		X			X		X
DXA Full Body	X		X			X		X
Fasting blood glucose	X		X			X		X
Fasting plasma insulin	X		X			X		X
HOMA-IR	X		X			X		X
PWV & PWA	X							
Echocardiography	X							

Study Phase	Observation			Treatment				
	Study Visit #1 (0 month)	Blood Draw Visit A (3 month)	Study Visit #2 (6 month)	Randomization (no visit)	Blood draw Visit B (7.5 month)	Study Visit #3 (12 month)	Blood Draw Visit C (13.5 month)	Study Visit #4 (18 month)
Anthropometry	X		X			X		X
QOL and Body Image questionnaires	X		X			X		X
Pubertal Status	X		X			X		X
Smoking status	X							
Fasting lipid panel: TG, TC, HDL-C, LDL-C	X		X			X		X
Non-HDL-C	X		X			X		X
HbA1C	X		X			X		X
PAI-1	X		X			X		X
Hs-CRP	X		X			X		X
IL-6	X		X			X		X
Adiponectin	X		X			X		X
Leptin	X		X			X		X
Lipoprotein subclass analysis	X		X			X		X
T4	X	X	X		X	X	X	X
TSH	X	X	X		X	X	X	X
ANTITPO		X						
ANTITG		X						
Physical activity armband (Worn for 7 days after study visit)	X		X			X		X
Randomization				X				

Dispense study medication (placebo or levothyroxine)				X				
Dispense levothyroxine – both groups						X		
Dietary intake	XXX		XXX			XXX		XXX
Drug Compliance					X	X	X	X
Adverse Events					X	X	X	X

FIGURE 1: STUDY DIAGRAM



1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

The AAP recommends yearly screening of thyroid studies in Down syndrome (DS)⁽¹⁾. Clinical experience suggests that TSH concentrations in the subclinical hypothyroid range (5-10 mIU/L) are not uncommon in DS, but the benefits and risks of treating subclinical hypothyroidism (SCH) in the DS population are not known. In adults, SCH has been associated with increased cardiometabolic risk and individuals with DS may be at increased cardiometabolic risk as well. **Thus, this preliminary study will explore the effects of levothyroxine treatment of subclinical hypothyroidism on cardiometabolic risk and quality of life in pre-adolescents and adolescents with DS. This is part of the larger study, IRB #9233, investigating cardiometabolic risk in the DS population.**

1.2 Name and Description of Intervention

Levothyroxine: Group 1 (delayed treatment) and Group 2 (immediate treatment) will receive a starting dose of 0.5-1 mcg/kg/day. Group 1 will receive levothyroxine treatment at the 12th month and continue treatment until the 18th month. Group 1 will receive a total of 6 months of levothyroxine treatment. Group 2 will receive levothyroxine treatment at the 6th month and continue treatment until the 18th month. Group 2 will receive a total of 12 months of levothyroxine treatment.

Placebo: Group 1 (delayed treatment) will receive placebo for the first 6 months.

Investigators will be unblinded to permit titration of levothyroxine doses. Dose adjustments will be made at the discretion of the PI. TSH will be repeated 6 weeks after any dose adjustment.

1.3 Findings from Non-Clinical and Clinical Studies

1.3.1 Non-Clinical Studies: N/A

1.3.2 Clinical Studies

1.3.2.1 Human Pharmacokinetics: N/A

1.3.2.2 Clinical Studies in Adults

Subclinical hypothyroidism in adults is associated with increased risk of incident ischemic heart disease and related mortality, an effect that may be attenuated by levothyroxine treatment⁽³⁾. The mechanisms linking subclinical hypothyroidism and CVD remain to be delineated but have been reviewed by Biondi and Cooper⁽⁴⁾. Studies of CVD risk in patients with subclinical hypothyroidism have shown: 1. arterial stiffness identified by pulse wave analysis was increased in adults⁽⁵⁾; 2. endothelial dysfunction was present^(6, 7), which improved with levothyroxine treatment⁽⁸⁾; 3. and elevated CRP was present in some⁽⁹⁻¹¹⁾ but not all⁽¹²⁾ studies. Data on lipogenic profiles were also conflicting: LDL and total

cholesterol^(10, 13, 14) were higher in some studies (an effect potentially mediated through insulin resistance⁽¹⁵⁾) but not in other studies^(12, 14). In an RCT, levothyroxine treatment didn't improve total cholesterol and LDL, but a sub-analysis suggested benefit for subjects with TSH>12 mIU/L or with elevated LDL⁽¹⁶⁾. Debate remains whether adults with subclinical hypothyroidism should be treated⁽¹⁷⁾.

1.3.2.3 Clinical Studies in Children

Data in children with subclinical hypothyroidism are limited. Despite the recommendations to screen for thyroid dysfunction, evidence to guide management of marginally elevated TSH (with normal T4) in children with DS is equally sparse. In non-DS children, TSH>4.65 mIU/L was associated with lower HDL⁽¹⁸⁾. One year of levothyroxine treatment in children with subclinical hypothyroidism and short stature improved growth velocity⁽¹⁹⁾. Left ventricular function and LV mass (by echocardiography) was not different in 16 children with DS and subclinical hypothyroidism (TSH>6.5 mIU/L; mean TSH = 7.8 mIU/L) vs. 25 children with DS and normal TSH. However, these findings may be limited by the small sample size. An intervention study of 7 subjects age 2-42 years with DS and "hypothyroidism" defined as low T4 and normal or elevated TSH (0.2-18.9 mIU/L) on 8 weeks of levothyroxine treatment did not improve developmental or functional outcomes. Anthropometrics and CMR factors were not examined⁽²⁰⁾. In contrast, increased TSH in the absence of overt congenital hypothyroidism is common in neonates with DS⁽²¹⁾ and prompted an RCT in 181 neonates with DS. TSH-directed levothyroxine treatment was associated with better growth, weight gain, and motor development after 24 months compared to placebo⁽²²⁾. These findings highlight that the "asymptomatic" component of subclinical hypothyroidism may have medically-relevant effects.

1.4 Selection of Drugs and Dosages

Levothyroxine (T4) is a synthetic form of thyroxine, an endogenous hormone secreted by the thyroid gland. T4 is converted to its active metabolite, L-triiodothyronine (T3). Thyroid hormones (T4 and T3) then bind to thyroid receptor proteins in the cell nucleus and exert metabolic effects through control of DNA transcription and protein synthesis. Thyroid hormones are involved in normal metabolism, growth, and development, promote gluconeogenesis, increase utilization and mobilization of glycogen stores, stimulate protein synthesis, and increase basal metabolic rate. Onset of action for the oral form of levothyroxine is 3-5 days. Oral tablets are available in a number of doses: 25 mcg, 50 mcg, 75 mcg, 88 mcg, 100 mcg, 112 mcg, 125 mcg, 137 mcg, 150 mcg, 175 mcg, 200 mcg, 300 mcg. In children, pills are frequently cut in half to further refine doses: 37.5 mcg, 44 mcg, 56 mcg, 62.5 mcg, 68.5 mcg. Younger children tend to require higher doses for body weight of thyroxine replacement than older children and adults with hypothyroidism (TSH >10 mIU/L).

Typical doses for individuals with hypothyroidism, based on age, are: age 6-12 year: 4-5 mcg/kg/day; age > 12 years: 2-3 mcg/kg/day; adults and in older children in whom growth is complete: 1.7 mcg/kg/day. For subclinical hypothyroidism, a starting dose of 0.5-1

mcg/kg/day is typical. For the purposes of this study, subjects will be initiated on a dose between 0.5-1 mcg/kg/day, depending on the available tablet formulations and the PI's medical discretion. TFTs will be checked 6 weeks after treatment initiation and titrated to achieve TSH 0.5-3 mIU/L range.

Generic lactase tablets will be used as placebo and will be dispensed by the CHOP Investigational Pharmacy.

1.5 Relevant Literature and Data

The AAP recommends yearly screening of thyroid studies in DS⁽¹⁾ and reports a 4-18% risk of hypothyroidism, with risk increasing with age. The TSH threshold for defining hypothyroidism varies among studies⁽²³⁾. Symptoms suggestive of hypothyroidism--constipation, dry skin, weight gain, and decreased growth velocity—are not uncommon in children with DS⁽²³⁾. Studies in DS suggest TSH is often higher than the normal population and occurs in the presence of normal thyroxine concentration, a phenomenon referred to as subclinical hypothyroidism or isolated hyperthyrotropinemia. The clinical relevance of isolated increased TSH is unknown and there is little data to guide clinical management. Rates of medically treated thyroid disease (>95% with thyroid hormone replacement) in children with DS enrolled in Tennessee Medicaid increased by 73% from 1995 to 2003 (22.37/1000 child years) subsequent to the 2001 AAP guideline publication. They estimated a 10.8% population based incidence of thyroid dysfunction in 1257 children with DS⁽²⁴⁾. The prevalence of overt hypothyroidism was not reported, and the question remains whether children with DS and subclinical hypothyroidism are being treated.

Subclinical hypothyroidism in adults is associated with increased risk of incident ischemic heart disease and related mortality, an effect that may be attenuated by levothyroxine treatment⁽³⁾. As described in section (1.3.3.1), CVD risk may be higher in adults with SCH⁽⁴⁻¹⁵⁾, and levothyroxine treatment may reduce CVD risk in adults with SCH⁽¹⁶⁾.

Subclinical Hypothyroidism in Children and DS

Data in children with subclinical hypothyroidism are limited. Despite the recommendations to screen for thyroid dysfunction, evidence to guide management of elevated TSH in children with DS is equally sparse. In non-DS children, TSH>4.65 mIU/L was associated with lower HDL⁽¹⁸⁾. One year of levothyroxine treatment in short children with subclinical hypothyroidism and short stature improved growth velocity⁽¹⁹⁾. Left ventricular function and LV mass (by echocardiography) was not different in 16 children with DS and subclinical hypothyroidism (TSH>6.5 mIU/L; mean TSH = 7.8 mIU/L) vs. 25 children with DS and normal TSH. However, these findings may be limited by the small sample size. An intervention study of 7 subjects age 2-42 years with DS and hypothyroidism, defined as low T4 and normal or elevated TSH (0.2-18.9 mIU/L) on 8 weeks of levothyroxine treatment did not improve developmental or functional outcomes. Anthropometrics and CMR factors were not examined⁽²⁰⁾. In contrast, increased TSH in the absence of overt congenital hypothyroidism is common in neonates with DS⁽²¹⁾ and prompted an RCT in 181 neonates with DS. TSH-directed levothyroxine treatment was associated with better growth, weight gain, and motor development after 24 months compared to placebo⁽²²⁾. These findings

highlight that the “asymptomatic” component of subclinical hypothyroidism may have medically-relevant effects. **This aim will provide potentially clinically relevant preliminary evidence for the treatment of subclinical hypothyroidism in DS.**

1.6 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 with all applicable Federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 54, 56, 312, 314 and 812 and the Good Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonisation (ICH). 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

2.1 Primary Aim

To determine the effect of levothyroxine replacement on non-HDL-C levels in pre-adolescents and adolescents with DS and subclinical hypothyroidism. Primary hypothesis: We hypothesize levothyroxine treatment of subclinical hypothyroidism will decrease non-HDL-cholesterol in DS

2.2 Secondary Aim

- 1) To explore the effect of levothyroxine replacement on small LDL-P, fat mass, BMI Z score, and inflammation in pre-adolescents and adolescents with subclinical hypothyroidism and DS. Hypothesis: We hypothesize thyroxine treatment of subclinical hypothyroidism will decrease small LDL-P, decrease fat mass, decrease BMI Z score, and decrease inflammation
 - 2) To determine the effect of levothyroxine replacement on QOL, lifestyle (physical activity, nutrition) and body image in pre-adolescents and adolescents with DS and subclinical hypothyroidism. Hypothesis: Treatment of subclinical hypothyroidism will be associated with improved QOL , physical activity, and body image in pre-adolescents and adolescents with DS and subclinical hypothyroidism.
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3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This randomized, single-blinded, intervention study will take place at the CHOP outpatient CTSC and at Children's National Medical Center. Subjects with Down syndrome and subclinical hypothyroidism (TSH: 5-10 mIU/L) ages 8-20 years will be recruited for a trial of levothyroxine treatment, using a switching replication design. All subjects will be observed for 6 months. TSH and T4 will be obtained at the 3rd and 6th month of the observation period. If a subject has a TSH which has reverted to normal at 3 months, they will continue on to the 6-month check. Only subjects with persistently elevated TSH (5-10 mIU/mL) at 6 months will be enrolled in the randomized phase. While there is no standard definition of "persistently elevated," in this study, we consider TSH level that is 5-10 mIU/L at 0 and 6 months as "persistently elevated." If TSH is < 5 mIU/L at 0 months and the subject has a history of TSH 5-10 mIU/L, a repeat TSH may be drawn, at the discretion of the principle investigator, within 3-6 months; if TSH is < 5 mIU/mL at 6 months, a repeat TSH may be drawn, at the discretion of the investigator, within 1-2 weeks. If the repeat TSH is < 5 mIU/L, the subject will not be eligible to continue. If TSH is > 10 mIU/L during the first 6 months, a repeat TSH will be drawn within 1-2 weeks. If the repeat TSH is > 10 mIU/L, thyroid dysfunction will not be considered subclinical. The subject will be referred to an endocrinologist for definitive therapy. After 6 months of observation, subjects will be randomized to: Group 1 (6 months of placebo, followed by 6 months of levothyroxine) or Group 2 (12 months of levothyroxine). The initial levothyroxine dose will be 0.5 – 1 mcg/kg/day, with a goal of TSH desired range of 0.5 – 3 mIU/L. Subjects/families will be blinded to the treatment arm. Investigators will be unblinded to permit titration of levothyroxine dose. TSH and T4 will be measured after 6 weeks (month 7.5) of levothyroxine/placebo commencement (as a measure of efficacy of levothyroxine dose), and then at month 12. At month 12, levothyroxine will be initiated in Group 1 (delayed treatment), allowing for 6 months of treatment. Group 2 (immediate treatment) will continue an additional 6 months of levothyroxine (12 months total). TSH will be checked at 6 weeks (month 13.5) and at month 18 in both groups. Dose adjustments will be made at the discretion of the PI. TSH will be repeated 6 weeks after any dose adjustments, which may be in addition to the time points above if necessary. This switching replication design will permit using each child to serve as his/her own control and provides a placebo control period for each treated child. The goal is for 20 subjects to complete the 18 month study.

3.1.1 Screening Phase

Potential subjects will be identified using the protocol inclusion and exclusion criteria. With their permission, subjects may be screened and recruited from Aim 1: Cardio risk factors in DS youth (IRB #12-009233), which is recruiting male and female subjects with Down syndrome in the age range of 10-20 years of age. Additional children will be recruited from local primary care pediatric practices and from the Division of Endocrinology where children with DS are routinely referred for thyroid function abnormalities. A telephone interview and medical record review to determine eligibility will be conducted on subjects who are not recruited from #9233.

3.1.2 Observation Phase

The first 6 months of the study are the Observation Phase of the study. Only subjects with persistently elevated TSH during the Observation Phase will proceed to the Treatment Phase. Subjects that have TSH >10 mIU/L during the 6 month Observational Phase will have a repeat TSH test within 1-2 weeks. If the repeat TSH is >10 mIU/mL, the subject not be considered to have subclinical hypothyroidism and will not qualify to continue the study. They will be referred to an endocrinologist for treatment. Subjects who have TSH <5 mIU/L at the 6 month visit may have a repeat TSH test within 1-2 weeks; this will be at the discretion of the study investigator. If TSH or repeat TSH (if applicable) is <5 mIU/L, the subject will be considered to have normal levels of TSH and will not qualify to continue the study. Subjects who have a history of subclinical hypothyroidism, but at the 0 month visit have a TSH value in the normal range, may have a repeat TSH test within 3-6 months; this will also be at the discretion of the study investigator.

Study Visit #1 (0 Month):- For subjects recruited from #9233, #9233 data will serve as Study Visit #1 data. Study visit #1 will not be necessary for subjects recruited from #9233.

Subjects who have not participated in Study #9233 will complete Study Visit #1. Subjects and their parents/guardians will come to the research facility following a 12 hour overnight fast. Parental/guardian/Legal Authorized Representative consent and child assent (if child is deemed capable of assent) will be obtained prior to any study procedures being performed. Females will receive a urine pregnancy test. Females found to be pregnant will be disqualified from the study and will receive appropriate counseling and referral. Study participants will have the following measures: fasting blood samples, TSH, T4, anthropometric measurements, whole body DXA scan, blood pressure, pubertal assessment, pulse wave velocity & pulse wave analysis, echocardiography and questionnaire assessments. After the study visit, subjects will be asked to wear a physical activity arm band at home for 7 days, and a 3 day dietary recall will be done by telephone.

Blood Draw Visit A(3 Month):- During this observation time frame, subjects will have a blood draw to check TSH and T4 levels. This blood draw can be done at the research facility or at other hospital-affiliated sites.

Study Visit #2 (6 Month): – Subjects and their parents/guardians will present to the research facility following an overnight fast. At this study visit, the subject will have the following measures: fasting blood samples, TSH, T4, anthropometric measurements, whole body DXA scan, blood pressure, pubertal assessment and questionnaire assessments. Females will receive a urine pregnancy test. Females found to be pregnant will be disqualified from the study and will receive appropriate counseling and referral. After the study visit, subjects will be asked to wear a physical activity arm band at home for 7 days, and a 3 day dietary recall will be done by telephone.

3.1.3 Treatment Phase

Randomization and blinding -

Only subjects with persistently elevated TSH (5 – 10 mIU/mL) will be randomized into the following 2 groups of 10: Group 1 (Delayed treatment) or Group 2 (Immediate treatment). A 3 month supply of placebo will be dispensed to subjects that have been randomized into Group 1 and a 3-month supply of levothyroxine will be dispensed to subjects randomized into Group 2.

Blood Draw Visit B (7.5 Month): TSH and T4 will be measured 6 weeks after the Study Visit #2 (approximately 7.5 months from the start of the study). Subjects will obtain a blood draw. Dose adjustments will be made for Group 2 as needed. Another 3-month supply of placebo or levothyroxine (dose adjusted as necessary) will be mailed to the home. Subjects requiring dose adjustments will have TSH and T4 in another 6 weeks to confirm/assure TSH is in the desired range.

Study Visit #3 (12 Month): – Subjects and their parents/guardians will present to the research facility following a 12-hour overnight fast. At this study visit, the subject will have the following measures: fasting blood samples, TSH, T4, anthropometric measurements, whole body DXA scan, blood pressure, pubertal assessment and questionnaire assessments. Females will receive a urine pregnancy test. Females found to be pregnant will be disqualified from the study and will receive appropriate counseling and referral. After the study visit, subjects will be asked to wear a physical activity arm band at home for 7 days, and a 3 day dietary recall will be done by telephone. TSH and T4 will be reviewed and levothyroxine will now be dispensed to both Groups 1 and 2. Group 2 will continue on current dose if TSH within range; otherwise dose adjustments will be made as necessary.

Blood Draw Visit C (13.5 Month): TSH and T4 will be measured 6 weeks after the 12 month visit (approximately 13.5 months from the start of the study). Subjects will have blood drawn. A subsequent blood draw 6 weeks later will be required for any dose adjustments.

Study Visit #4 (18 Month): – Subjects and their parents/guardians will present for a final follow up at research facility following a 12-hour overnight fast. At this study visit, the subject will have the following measures: fasting blood samples, TSH, T4, anthropometric measurements, whole body DXA scan, blood pressure, pubertal assessment and questionnaire assessments. Females will receive a urine pregnancy test. Females found to be pregnant will be disqualified from the study and will receive appropriate counseling and referral. After the study visit, subjects will be asked to wear a physical activity arm band at home for 7 days, and a 3 day dietary recall will be done by telephone.

3.2 Allocation to Treatment Groups and Blinding

Randomization and allocation concealment: Children will be randomized to two groups. Both will be observed for 6 months. One will be switched to the intervention at 6 months, and continued thereafter. The other will be switched to placebo at 6 months and to the intervention at 12 months. Randomization will be performed centrally by the project statisticians and implemented using opaque sealed envelopes to ensure allocation concealment. Balance between treatment arms will be maintained by use of randomly permuted blocks with unequal block sizes and with stratification by patient gender (two

strata, male and female and age (two strata, ages 8-14 and ages 14-20). After envelopes are opened, treatment assignment will be blinded to the subjects (and their parents).

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study Participation

The total study duration per subject will be between 6 to 18 months.

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

The study will be conducted at 2 study sites, The Children's Hospital of Philadelphia and Children's National Medical Center. Interim blood draws may take place at satellite Children's Hospital of Philadelphia and Children's National Medical Center sites, if it is more convenient for the family.

It is expected that approximately 29 subjects will be enrolled to yield 20 study completers.

3.4 Study Population

3.4.1 Inclusion Criteria

Inclusion Criteria

1. Males and females, ages 8 – 20 years
2. Diagnosis of Down syndrome
3. Subclinical hypothyroidism: TSH level between 5 – 10 mIU/L, normal T4
4. Parental/guardian permission (informed consent) and if appropriate, child assent
5. Females who are ≥ 11 years of age or who are menarchal must have a negative urine/serum pregnancy test
6. Committed to adherence to levothyroxine treatment and study completion

3.4.2 Exclusion Criteria

Exclusion Criteria

1. Pregnancy
2. Type 1/Type 2 diabetes
3. Chronic medical conditions or medication use that can affect growth, nutrition, blood glucose, insulin secretion, or thyroid function (such as lithium or certain seizure medications)
4. Current use of levothyroxine or anti-thyroid hormone
5. Cyanotic congenital heart disease, or pulmonary hypertension (as described by last echo report in subjects with CHD), or congenital heart disease considered medically unstable by the study cardiologists

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 Screening Phase

4.1.1 Telephone screening and medical record review

A telephone screening and medical record review will be done to assess eligibility for the study.

4.2 Observation Phase

4.2.1 Study Visit #1 (0 Month), Blood Draw Visit A (3 Month) & Study Visit #2 (6 Month)

The purpose of the 3 Observation Phase visits is to observe TSH and T4 levels over a period of 6 months. All subjects will get a blood draw for TSH and T4 labs 3 months after the Study Visit #1. The TSH and T4 at 0 and 6 months will be done as part of Study Visit #1 and #2. At the PI's discretion, additional blood draws following the 0 and 6 month visits may take place to assess TSH levels.

The following visits and procedures occur during the Observation Phase:

Study Visit #1 (0 month) = 1 visit lasting approximately 4.5 hours

- 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
- Vital Signs: 10 minutes
- DXA scan: 10 minutes
- Anthropometric measurements: 15 minutes
- PWV and PWA: 30 minutes
- Echocardiography: 20 minutes
- Family history: 10 minutes
- Questionnaire assessments (PedsQL, IWQOL, BES, FRS, ASAQ, CEBQ, CFQ, ABAS-II, HFI): 1.5 hours, or as long as needed by subject and parent/guardian. HFI will be mailed to family and completed prior to visit.
- Physical activity armband (worn at home for 7 days)
- 3 day dietary recalls over the phone from home = Approximately 30 minutes for each diet recall.

Blood Draw Visit A (3 month) = Approximately 15 minutes for blood draw

- TSH & T4 blood draw: 15 minutes

Study Visit #2 (6 month) = 1 visit lasting approximately 4 hours

- Fasting blood draw: 15 minutes
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- Pubertal Status Exam: 5 minutes
 - Vital Signs: 10 minutes
 - DXA scan: 10 minutes
 - Anthropometric measurements: 15 minutes
 - Family history: 10 minutes
 - Questionnaire assessments (PedsQL, IWQOL, BES, FRS, ASAQ, CEBQ, CFQ, ABAS-II, HFI): 1.5 hours, or as long as needed by subject and parent/guardian. HFI will be mailed to family and completed prior to visit.
 - Physical activity armband (worn at home for 7 days)
 - 3 day dietary recalls over the phone from home = Approximately 30 minutes for each diet recall.

4.3 Treatment Phase

4.3.1 Randomization

After Study Visit #2 (6 months), only subjects with persistently elevated TSH (5-10 mIU/mL) will be randomized into two groups of 10. Randomization will be performed centrally by the project statisticians and implemented using opaque sealed envelopes to ensure allocation concealment. Balance between treatment arms will be maintained by use of randomly permuted blocks with unequal block sizes and with stratification by patient gender (two strata, male and female) and age (two strata, ages 8-14 and ages 14-20). After envelopes are opened, treatment assignment will be blinded to the subjects and their parents/guardians.

The following visits and procedures will occur during the Treatment Phase:

Blood Draw Visit B (7.5 month): Approximately 15 minutes for blood draw

TSH and T4 blood draw will be done 6 weeks after the 6 month visit (at 7.5 months) as a measure of efficacy of levothyroxine dose.

Study Visit #3 (12 month) = 1 visit lasting approximately 4 hours

- Fasting blood draw: 15 minutes
- Pubertal Status Exam: 5 minutes
- Vital Signs: 10 minutes
- DXA scan: 10 minutes
- Anthropometric measurements: 15 minutes
- Family history: 10 minutes
- Questionnaire assessments (PedsQL, IWQOL, BES, FRS, ASAQ, CEBQ, CFQ, ABAS-II, HFI): 1.5 hours, or as long as needed by subject and parent/guardian. HFI will be mailed to family and completed prior to visit.
- Physical activity armband (worn at home for 7 days)
- 3 day dietary recalls over the phone from home = Approximately 30 minutes for each diet recall.

Blood Draw Visit C (13.5 month): Approximately 15 minutes for blood draw

TSH and T4 blood draw will be done 6 weeks after the 12 month visit (at 13.5 months) as a measure of efficacy of levothyroxine dose.

Study Visit #4 (18 month) = 1 visit lasting approximately 4 hours

- Fasting blood draw: 15 minutes
- Pubertal Status Exam: 5 minutes
- Vital Signs: 10 minutes
- DXA scan: 10 minutes
- Anthropometric measurements: 15 minutes
- Family history: 10 minutes
- Questionnaire assessments ((PedsQL, IWQOL, BES, FRS, ASAQ, CEBQ, CFQ, ABAS-II, HFI): 1.5 hours, or as long as needed by subject and parent/guardian. HFI will be mailed to family and completed prior to visit.
- Physical activity armband (worn at home for 7 days)
- 3 day dietary recalls over the phone from home = Approximately 30 minutes for each diet recall.

4.4 Blood Draw Visits B & C

Randomized subjects will be asked to have a blood draw to check TSH and T4 levels 6 weeks after the 6 month and 12 month visits. TSH will be repeated 6 weeks after any dose adjustment. The purpose to for this check is to adjust the levothyroxine dose if the PI deems necessary.

4.5 Concomitant Medication

After the participant has completed the screening and interim visits, the participant will be randomized to take an oral placebo or levothyroxine treatment. The initial levothyroxine dose will be 0.5-1 mcg/kg/day. Participants are required to bring in their full or partially full bottles of placebo/levothyroxine to each study visit (Study Visits #3 and 4). Subjects can continue other medications; these will be recorded at each study visit.

4.6 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 Investigator for lack of adherence to study treatment, visit schedules, or AEs. The Investigator or the Sponsor (if applicable) 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 Investigator becomes 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

- Fasting blood draw: This will be completed by nursing staff. A total of approximately 12.5 mL (approximately 2½ teaspoons) of blood will be obtained.
 - Pubertal Status Exam: Tanner staging of puberty will be performed by a pediatric endocrinologist team member. This exam will take place in a private exam room.
 - Vital Signs: Blood pressure, heart rate, and respiratory rate will be taken by the nursing staff. The blood pressure will be taken 3 times and averaged. Vital signs will be collected after the blood draw.
 - Full Body DXA Scan: The DXA scan will be performed by trained . The scan measures BMC, BMD, BMD-Z, fat mass, lean body mass, total mass, fat-free mass and percent fat.
 - Anthropometry: Weight will be measured by digital electronic scale (Scaletronix) and stature on a stadiometer (Holtain). Body proportions and fat distribution measurements (sitting height, arm and waist circumference, and skin fold thickness at triceps, biceps, subscapular, and suprailiac sites) will be measured.
 - Pulse Wave Velocity (PWV) and Pulse Wave Analysis (PWA): Aortic PWV and PWA will be measured by a trained technician. PWV and PWA will only be done at Study Visit #1.
 - Echocardiography: LV mass will be measured by echocardiography by a trained technician. Echocardiography will only be done at Study Visit #1.
 - Family History: The study coordinator/appropriate member of the investigative team will administer the CRF to collect relevant patient medical history, family history of CVD/dyslipidemia/T2DM.
 - Questionnaire Assessments: The following questionnaires: (PedsQL, IWQOL, BES, FRS, ASAQ, CEBQ, CFQ, ABAS-II, body satisfaction scales) will be administered by the study coordinator/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
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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 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.
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- 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.
 - Sense Wear® Armbands (Body Media, Inc): Participants will pick up the Sense Wear armbands at the visit. The subject will be asked to wear the armband accelerometer for 7 consecutive days, 24 hours per day. The exception of wearing the armband is when the subject is bathing/showering or if participating in water activities, such as swimming. Information collected will include the amount and intensity of physical/sedentary activities and sleep.
 - 3 day dietary recalls: After the study visit, a nutritionist will contact the participant and guardian by phone to collect information as to what the participant ate and drank within the last 24 hours. This will be done three times (2 weekdays and 1 weekday).

5.1.1 Laboratory Evaluations

A fasting blood sample will be performed for the laboratory evaluations described in section 5.1.1.1.

5.1.1.1 Table: Clinical Laboratory Tests

Tests	
Lipid panel (TG, TC, HDL-C, LDL-C)	Measured at CHOP Clinical Lab
Non-HDL cholesterol	Calculated as Total cholesterol – HDL-C from the lipid

	panel
Lipoprotein subclass analysis	By LipoScience, Inc. (Raleigh, NC) using NMR spectroscopy
Blood glucose	Measured in duplicate at study site on NOVA Biomedical STAT strip meter
Plasma immunoreactive insulin	Measured in duplicate by ELISA in the CHOP CTRC Biochemistry Core Lab
Insulin resistance	Fasting insulin and glucose used to calculate Homeostasis Model Assessment-Insulin Resistance Index (HOMA-IR): [fasting insulin (uIU/mL) x fasting BG(mmol/L)]/22.5
Thyroglobulin antibody	Measured at CHOP Clinical Lab
Thyroid peroxidase antibody	Measured at CHOP Clinical Lab
High sensitivity C-reactive protein (hs-CRP)	Measured in the Penn CTRC TCL
Interleukin 6 (IL-6)	Measured at CHOP CTRC Biochemistry Core Lab using R&D kits
PAI 1	Measured at CHOP CTRC Biochemistry Core Lab using R&D kits
HbA1c	Measured at CHOP Clinical Lab
Adiponectin & Leptin	Both measured in duplicate at CHOP CTRC Biochemistry Core Lab using Alpco kits
TSH & T4	Measured at CHOP Clinical Lab

5.1.1.2 Pregnancy Testing

A urine pregnancy test will be performed for female subjects who are ≥ 11 years old or who are menarchal during the following visits: screening/baseline, 6 month, 12 month, and 18 month. 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.

5.2 Safety Evaluation

Subject safety will be monitored by adverse event reporting, vital signs, physical examinations, and TSH levels. This protocol involves a very small radiation exposure, which is unlikely to cause any untoward effects to the participant. However, because the protocol may be unsafe to an unborn child, pregnancy is an exclusion criterion of the study.

6 STATISTICAL CONSIDERATIONS

6.1 Statistical Methods

6.1.1 Baseline Data

Data management will proceed as with Aim 1, with particular attention to obtaining all information on each subject at each encounter. Subject retention in our previous studies of children with DS has been excellent.

Estimates will be based on a standard mixed effects piecewise longitudinal data model⁽²⁾, implemented in the Stata v 12 program “xtmixed”, to estimate in a single model both longitudinal (within subject) and cross-sectional (across subject) effects of the intervention. Cross-sectional comparisons will be possible for the second 6-month period of observation when ½ of subjects will be switched to treatment while the other ½ will take a placebo. A piecewise model allows for a change in trajectory of an endpoint upon start of the intervention and for a possible shift in pattern of endpoint values soon after intervention, thus allowing for contrasts of both levels and trajectories. After descriptive statistics, mixed effects linear regression, with random intercepts for subject-specific baseline z-score, and random slopes (for inter-subject variation in trajectories over time), will model and compare outcomes over time. Inclusion of quadratic terms for time, and implementation of splines, will avoid unrealistic assumptions of linear changes in outcomes^(25, 26). Baseline covariates and auxiliary variables that predict dropout will be included to adjust for any residual imbalance and to meet the model assumption of dropout at random. Multiple imputations will be used for any missing covariates⁽²⁷⁾.

Primary comparisons will assume complete treatment adherence. Using the methods of Nagelkerke⁽²⁸⁾ and Small⁽²⁹⁾, we will estimate the effect of the intervention among those subjects who adhered to treatment. Robust confirmatory analysis will use generalized estimating equations weighted to adjust for dropout⁽³⁰⁾.

Key contrasts will compare the level of each endpoint before and after treatment by comparing differences of model-based expected values at the end of treatment and expected values at baseline; expected values arise from implementation of mixed effects models. This approach permits adjustment for relevant changes in patient characteristics over time (e.g. changes in growth and pubertal status) and for any baseline factors predicting dropout. Alternative contrasts will compare slopes of endpoints over time prior to the intervention against the slopes after treatment initiation. Pre-intervention measures will improve statistical power.

Sensitivity analyses will examine the potential impact of informative dropout⁽³¹⁾, although this source of bias will be minimized through subject retention and follow-up after dropout⁽³²⁾. Supplementing models will be complete descriptive reports, including graphical displays of cholesterol and BMI-Z trajectories for each subject.

Potential Problems/Feasibility: Dosing of levothyroxine treatment tends to be weight-based and is not an exact science. The starting daily dose of 0.5-1 mcg/kg/day is less than

full replacement doses since TSH will be in the 5-10 mUI/mL range and we wish to avoid “over-supplementing” and suppressing TSH. TSH will be rechecked 6 weeks after levothyroxine initiation to confirm TSH is in the desired range. After 6 weeks some subjects may not have “optimal” TSH levels and dose adjustments will be required. We expect that by 3 months following levothyroxine initiation, all subjects will have TSH in the desired range, allowing an additional 3 months for the effect of intervention to be realized; in fact, the immediate treatment group will be followed for 12 months, over which time effects upon body composition and CMR factors should be apparent. This study might find that the relationship between subclinical hypothyroidism and CMR factors is pure association, an important finding as the medical community looks for evidence upon which to base management of “mild” TSH elevations in DS. In fact, this finding could avert unnecessary treatment of subclinical hypothyroidism in adolescents with DS.

6.1.2 Efficacy Analysis

Key contrasts will compare the level of each endpoint before and after treatment by comparing the differences of model-based expected values at the end of treatment and expected values at baseline. These expected values will arise out of the implementation of mixed effects models that will adjust for any relevant changes in patient characteristics over time (such as changes in growth and pubertal status) as well as for any baseline factors that predict dropout. Alternative contrasts will compare the trajectories (slopes) of endpoints over time prior to the intervention against the slopes after the start of treatment.

6.1.3 Safety Analysis

To monitor for safety, data will be made available to investigators every 3 months. Since the PI will not be blinded to treatment arm and will be making dose adjustments, TSH data will be available to the PI as soon as it is available.

6.2 Sample Size and Power

Target sample size is 20, but 29 subjects will be enrolled to allow for dropout. Assuming that the dependent variables are continuous, the power calculations using nonparametric tests that would be suitable for both continuous and ordered data are listed below:

Correlation over time	Effect size of interaction (SD)	Power
0.5	0.7	0.84
0.6	0.6	0.71
0.8	0.4	0.82

6.3 Interim Analysis

Interim efficacy or safety analyses will be done to monitor TSH levels in all subjects. Subjects with persistently elevated TSH (5-10 mIU/mL) will be randomized for the

treatment phase. If TSH is >10 , a repeat test will be done within 1-2 weeks. If TSH remains >10 , the subject will not be randomized. Subjects that have TSH <5 may have a repeat TSH test within 1-2 weeks; this will be at the discretion of the study investigator. If TSH or repeat TSH (if applicable) is <5 , the subject will be considered to have normal levels of TSH and will not qualify to continue the study.

7 STUDY MEDICATION (INTERVENTION)

7.1 Description

Levothyroxine sodium tablets, USP contain synthetic crystalline L-3,3',5,5'-tetraiodothyronine sodium salt [levothyroxine (T₄) sodium]. Synthetic T₄ is identical to that produced in the human thyroid gland. 50 mcg levothyroxine tablets will be prescribed for anticipated doses ranging from 25 – 100 mcg/day (0.5-1 mcg/kg/day).

The placebo is generic lactase manufactured by Major Pharmaceuticals. Each caplet contains 3,000 FCC units of lactase enzyme (active ingredient). Inactive ingredients are: crospovidone, dicalcium phosphate, magnesium stearate, mannitol, microcrystalline cellulose, polyalditol, silicon dioxide, sodium citrate.

7.1.1 Packaging

A 3 month supply of tablets will be packaged in a child safe prescription container.

7.1.2 Labeling

Both levothyroxine and the generic lactase tablets are available commercially and will be labeled appropriately by the manufacturer. The investigational pharmacy will transfer a 3-month supply of tablets into a child-resistant vial and label with a patient-specific label that complies with all the federal, state and CFR requirements.

7.1.3 Dosing

Levothyroxine daily dose of 0.5 – 1 mcg/kg/day by mouth once per day.

7.1.4 Treatment Compliance and Adherence

Pill counts, monthly calendars for charting, and completion of a Supplement Adherence Questionnaire, a semi-structured interview, will be used to assess treatment compliance. Study staff will contact the families weekly to review adherence. The study psychologist will address with families issues that may be interfering with adherence.

7.1.5 Drug Accountability

Adequate records of study drug receipt and disposition will be maintained by the CHOP Investigational Pharmacy. The purpose of these records is to ensure regulatory authorities and the Sponsor that the investigational new drug will not be distributed to any person who is not a study subject under the terms and conditions set forth in this protocol. The study medication is to be prescribed by the Investigator or designee and may not be used for any purpose other than that described in this protocol. At study completion, all drug supplies including partially used and empty containers must be returned to the Study Coordinator.

8 SAFETY MANAGEMENT

8.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study. Following randomization, adverse events will be monitored using: (a) Home diary of adverse events (AE), rated by intensity (mild, moderate, severe). (b) Interview at each study visit to document intensity and frequency of AE.

8.2 Adverse Event Reporting

The Investigator is responsible for recording and reporting unanticipated problems related to research that occur during and after study treatment. All on-site SAEs will be reported to the IRB in accordance with IRB policies. AEs that are not serious will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

8.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

8.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,
- a life-threatening event (at risk of death at the time of the event),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity, or
- a congenital anomaly/birth defect in the offspring of a subject.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A distinction should be drawn between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.4.1 Relationship of SAE to study drug or other intervention

The relationship of each SAE to the study intervention should be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

8.5 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator will promptly notify the IRB of all on-site unanticipated, serious Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly. Written reports will be filed using the eIRB system and in accordance with the timeline below. External SAEs that are both unexpected and related to the study intervention will be reported promptly after the investigator receives the report.

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

8.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

8.6 Investigator Reporting of a Serious Adverse Event to Sponsor

Reporting must be consistent with regulatory, sponsor or GCRC requirements (if applicable)

8.7 Medical Emergencies

Medical emergencies that might develop during the course of the study would be referred to the patient's local emergency room.

9 STUDY ADMINISTRATION

9.1 Treatment Assignment Methods

9.1.1 Randomization

Only subjects with persistently elevated TSH (>5mIU/mL) at 6 months will be recruited for the randomization phase. After 6 months of observation, subjects will be randomized to one of two treatment arms.

- 1) 6 months of placebo followed by 6 months of levothyroxine
- 2) 12 months of levothyroxine

9.1.2 Blinding

Subjects/families will be blinded to treatment arm. Investigators will not be blinded to permit titration of levothyroxine dose.

9.1.3 Unblinding

At the conclusion of the study, subjects/families will be notified of the treatment arm they received.

9.2 Data Collection and Management

The CHOP CTIC Informatics Core will create case report forms (CRF) and a REDCap database for data capture. REDCap is an NIH-supported web-based data management software designed by Vanderbilt University investigators. Using REDCap checking tools, the BDMC will confirm completeness and valid values and will inform the investigative team of any errors or omissions for prompt resolution. Data entered in REDCap will be exported for use with Stata and SAS statistical packages as needed for immediate access to the research team.

The PIs are responsible for the accuracy and completeness of data collection and management. The PIs 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 (CHOP Outpatient CTIC) or with the study investigative staff. CRFs will be kept in a locked filing cabinet in a locked room at all times.

All information will be transferred to a REDCap password-protected database, located on a secure sever 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 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.

9.3 Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy and the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study.

No identifiable data will be used for future study without first obtaining IRB approval. The investigator will obtain a data use agreement between the provider (the PI) of the data and any recipient researchers before sharing a limited dataset (PHI limited to dates and zip codes).

9.4 Regulatory and Ethical Considerations

9.4.1 Data and Safety Monitoring Plan

Study progress and safety will be reviewed quarterly (and more 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 95th percentile for age, gender, and height. We will notify the parent/guardian/primary care provider with clinically relevant abnormal labs. Specifically, these include: lipid levels/insulin levels/blood glucose levels /TSH and T4. Note that some labs, such as lipids and insulin, are run in batches and reporting of results may be delayed.

Data Monitoring Board (DSMB) -- Interim monitoring and analysis

A data safety monitoring board (DSMB) will be established: (1) to protect study patients, (2) to safeguard their interests, (3) to monitor the overall conduct of the trial, (4) to help to protect the integrity of the trial, and (5) to supervise the conduct of any interim analyses. Specific rules and protocols for all reviews will be established in a formal protocol, and approved by the DSMB, prior to the first DSMB meeting. The DSMB will work jointly with the trial statisticians and clinical investigators to establish specific criteria to accomplish its tasks. The DSMB will consist of at least four members including a biostatistician, a developmental pediatrician from outside Children's Hospital of Philadelphia, a pediatric endocrinologist, and a senior faculty member with experience in clinical research and in care of children and adolescents with complex chronic illness. The DSMB will choose its chair.

The Project PI will establish procedures for identifying classes of AEs, documenting them on an Adverse Experience Report (AER) form, and then reporting them regularly to the DSMB(33) .

The DSMB will meet annually to review efficacy issues and adverse events, and will have additional conference calls as needed. Annual reports will be prepared for them by the CHOP Data Management Core. The DSMB will independently evaluate whether adverse events constitute grounds to discontinue the study.

The DSMB will report to the trial principal investigators within one month of the start of the DSMB meeting, or earlier if patient safety becomes an issue. Early stopping determinations will be limited to considerations of safety and adverse events; owing to the small sample size, there will be no formal early stopping rule for superiority or futility.

DSMB reporting; Independence of DSMB and study trial leadership

All data and the interim analyses will be conducted by the DSMB and the project statisticians, independently from the trial leadership and staff. The DSMB report will consist of the following elements:

DSMB monitoring plan and summary of report
Major protocol changes
Study accrual by month
Any violations of eligibility
Demographics
Adherence to medication
Dropout and loss to follow up
Analysis of endpoints for safety
Report of any adverse events
Formal recommendation on early stopping
Recommendations for notification to IRB

It will be the responsibility of the trial leadership to notify the IRB or IRBs involved of any issues that are relative to patient safety or to early stopping of the study.

9.4.2 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.

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
- Anthropometric measurements and pubertal status exam pose 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 pose 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.

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 μ Sv (or 1 mrem). This total amount of radiation is less than the exposure to daily background radiation at sea level (3,000 μ Sv 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 a pediatric endocrinologist 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.

Protection against risks associated with thyroid hormone treatment

Children and adolescents with subclinical hypothyroidism are commonly treated with thyroid hormone replacement, even in the absence of data supporting treatment. Similarly, subclinical hypothyroidism is frequently treated in the setting of DS despite a dearth of evidence to support or negate intervention. The primary risk of thyroid hormone treatment in this 6 month observation followed by 6-12 month intervention study is excessive replacement leading to relative hyperthyroidism with TSH suppression; short-term symptoms include restlessness, irritability, tachycardia, palpitations, poor sleep, and weight loss. To minimize these risks:

- The family will be instructed to observe and report these symptoms/signs to the study team
 - TSH and T4 will be checked if signs/symptoms develop
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- TSH and T4 will be checked 6 weeks after levothyroxine initiation, the time required to reach steady state; the dose will be titrated to maintain TSH in the normal range
 - If the initial TSH is within range, TFTs will be checked in another 14 weeks, sooner if symptoms arise
 - If a TSH is elevated or suppressed, the dose will be adjusted and TFTs repeated in 6 weeks to reassess thyroid status

The dose of levothyroxine replacement (0.5-1 mcg/kg/dy) is not expected to cause TSH suppression and symptoms of hyperthyroidism. A dose of 1 mcg/kg/day is used in the treatment of subclinical hypothyroidism in adults and is much lower than typical full replacement doses of 4-5 mcg/kg/day for age 6-12y; 2-3 mcg/kg/day for age >12 y but growth and puberty incomplete; and 1.7 mcg/kg/day for children in whom growth and puberty are complete. In fact, chronic suppressive doses of levothyroxine are routinely used following thyroidectomy and radioactive iodine ablation for thyroid cancer in adults and children. For children, this treatment means TSH is maintained in the low range (TSH < 0.1 mIU/L).(34); the major concern is the impact of years of suppressive therapy upon bone health. In this study, TSH will be measured and dose adjustments made for low TSH; thus, any subclinical hyperthyroidism induced by levothyroxine therapy will be brief. Morbidity from acute levothyroxine intoxication requires massive doses of levothyroxine (> 5-10 mg) and is not anticipated. The study team will remind families that, as with all medications, administration should be supervised by a parent and medications maintained out of reach of children.

The study team will review potential side effects and toxicities with subjects by telephone 4 weeks after treatment initiation. If symptoms are present, TSH and T4 will be checked and dose adjustments made if necessary. If symptoms are absent, TSH and T4 will be checked as scheduled and symptoms reviewed at 6 weeks. All symptoms will be recorded. The Investigative team will have immediate access to TSH and T4 when these data are available from the lab. Thyroid function data will also be reviewed monthly by the study team, to assess study dosing practice.

For subjects enrolled in this study, any subject who develops TSH > 10 mIU/L during the course of the study will have a repeat TSH test done within 1-2 weeks. If the repeat TSH remains > 10 mIU/mL, the subject will be withdrawn from the study and referred to endocrinology for management of hypothyroidism. If a suppressed TSH (TSH < 0.1 mIU/L) is identified in a child on placebo, the TSH will be repeated within 2-3 weeks. If the TSH remains suppressed the subject will be referred to endocrinology for management of possible hyperthyroidism. If a suppressed TSH (TSH < 0.1 mIU/L) is identified in a child on levothyroxine, the levothyroxine dose will be decreased and the lab repeated within 4-6 weeks. If the TSH remains suppressed or child has symptoms consistent with hyperthyroidism, the TSH will be discontinued and the TSH repeated in 6 weeks. If the TSH remains suppressed the subject will be referred to endocrinology for management of possible hyperthyroidism,

At the completion of the study, serum TSH and T4 will be measured, thyroid hormone replacement will be discontinued, and thyroid hormone results from the study reported to the PMD. The subject will be directed to follow-up with the primary care provider or with pediatric endocrinology to determine if continued thyroid hormone replacement is appropriate.

9.4.3 Potential Benefits of Trial Participation

There may be no direct benefit and benefits will be to future patients, science, or society. The significant direct potential benefit from participation in this trial would be that subjects found to have subclinical hypothyroid disease (elevated TSH >5-10 mIU/mL) will be treated with levothyroxine. If a subject's TSH is > 10 mIU/L during the observation period, a repeat TSH test will be done within 1-2 weeks. If the repeat TSH is >10 mIU/mL, the thyroid dysfunction will not be considered subclinical and the subject will be referred to an endocrinologist for definitive therapy. Participants may indirectly benefit from identification of abnormalities such as diabetes, 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 will be referred appropriately for further management and treatment.

9.4.4 Risk-Benefit Assessment

The benefits to participation in this trial outweigh the potential risks. Levothyroxine is used in treating subclinical hypothyroidism with few side effects.

9.5 Recruitment Strategy

Subjects from the corresponding study, IRB #9233 who consent to be screened for this study (IRB #9578) may be recruited if they meet eligibility criteria based on the information collected at the #9233 study visit. Subjects may also be recruited from CHOP's Pediatric Research Consortium (PeRC) and Recruitment Enhancement Core, the CHOP Clinical Research Finder, CNMC Primary Care sites, Endocrinology and Trisomy 21 Clinics, Cardiology Clinics, DS community events, other studies, and through local advertisements. The NIH DS-Connect recruitment will e-mail registry participants the IRB-approved recruitment flyer.

9.6 Informed Consent/Assent and HIPAA Authorization

For subjects recruited from IRB #9233:

The first #9578 visit for these subjects recruited from IRB #9233 will be Blood Draw Visit A. Prior to Blood Draw Visit A, verbal consent from the parent/guardian/legal authorized representative will be obtained and documented by the study coordinator/study investigator. A description of the procedures involved in Blood Draw Visit A, 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. Assent may not be obtained for screening purposes if the child subject is not available at the time of the phone call or is not cognitively able.

If the subject is eligible to continue to Study Visit #2, written informed consent for the entire study will be obtained prior to conducting Study Visit #2 procedures. The written informed consent process will be the same as described below for subjects who are not recruited from IRB #9233.

For subjects not recruited from IRB #9233:

Subjects not recruited from IRB #9233 will have an initial telephone screening interview; those who are eligible based on the study inclusion/exclusion criteria will be invited to participate.

Prior to conducting the screening interview, verbal consent from the 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. Assent may not be obtained for screening purposes if the child subject is not available at the time of the phone call or is not cognitively able.

Prior to conducting the study visit procedures, written informed consent will be obtained from the parents/guardians of children and adolescents with DS. Assent will be obtained from children who, by parent report, are at a first grade or 6 year level of development or higher. . Study participants will be shown a picture book that depicts the study visit and at-home procedures. If the study participant is able to communicate that they understand the procedures, then written assent will be obtained if the participant is able to write, and verbal assent will be obtained if they are not able to write. Assent will not be obtained from subjects who do not have sufficient capacity.

The consent/assent process will take place in a private consent 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).

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

For the screening and telephone verbal consent processes, consent will not be obtained for Down syndrome subjects 18-20 years of age.

Consent will be obtained from Down syndrome subjects aged 18-20, when possible, during the in-person written informed consent process that will occur at either Study Visit #1 or Study Visit #2. 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 understand if thyroid pill treatment will help improve the health of children
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and adolescents who have DS and SCH. 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.

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.

9.6.2 Waiver of Documentation of Consent

Waiver of documentation of consent is being sought for screening procedures, as the parent/guardian/legal authorized representative may be contacted by telephone and obtaining a signature will not be feasible. As described in (9.6), verbal consent will be obtained and documented by the study coordinator/study investigator prior to conducting the screening interview.

9.6.3 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

9.7 Payment to Subjects/Families

All study procedures and expenses will not be billed to the participant. The total compensation can be up to \$815 for the entire study.

Study Visit #1 (0 month): The total reimbursement for Study Visit #1 is \$150. The parent/guardian will receive a total of \$100 in a pre-paid bankcard to offset the burdens of transportation/parking/time off work/babysitting fees/meals. The bankcard will be loaded with \$50 immediately after the study visit and with an additional \$50 once all post-visit procedures are complete. The participant will receive a \$50 gift card for their efforts toward the study.

Blood Draw Visit A (3 month): For Blood Draw Visit A, the subject will receive \$25 in a pre-paid bankcard for their time and efforts.

Study Visit #2 (6 month): The total reimbursement for Study Visit #2 is \$105. The parent/guardian will receive a total of \$75 in a pre-paid bankcard to offset the burdens of transportation/parking/time off work/babysitting fees/meals. The bankcard will be loaded with \$50 immediately after the study visit and with an additional \$25 once all post-visit procedures are complete. The participant will receive a \$25 gift card plus a small gift worth \$5.00 for their efforts toward the study.

Blood Draw Visit B (7.5 month): For Blood Draw Visit B, the subject will receive \$25 in a pre-paid bankcard for their time and efforts.

Study Visit #3 (12 month): The total reimbursement for Study Visit #3 is \$105. The parent/guardian will receive a total of \$75 in a pre-paid bankcard to offset the burdens of transportation/parking/time off work/babysitting fees/meals. The bankcard will be loaded with \$50 immediately after the study visit and with an additional \$25 once all post-visit procedures are complete. The participant will receive a \$25 gift card plus a small gift worth \$5.00 for their efforts toward the study.

Blood Draw Visit C (13.5 month): For Blood Draw Visit C, the subject will receive \$25 in a pre-paid bankcard for their time and efforts.

Study Visit #4 (18 month): The total reimbursement for Study Visit #4 is \$105. The parent/guardian will receive a total of \$75 in a pre-paid bankcard to offset the burdens of transportation/parking/time off work/babysitting fees/meals. The bankcard will be loaded with \$50 immediately after the study visit and with an additional \$25 once all post-visit procedures are complete. The participant will receive a \$25 gift card plus a small gift worth \$5.00 for their efforts toward the study.

Additional TSH & T4 Checks: In the case of dose adjustments or TSH >10, subjects will receive \$25 in a pre-paid bankcard for each time they have their blood drawn to check TSH and/or T4 levels. Up to 3 TSH and T4 checks may be necessary in the event of dose adjustment(s) or TSH > 10. Subjects will receive up to \$75.00 for these TSH & T4 checks.

In rare circumstances, participants may not be able to complete all study procedures on the date of their study visit (for example, if a machine is broken). If this occurs, we would like to compensate the parent/guardian with an additional \$50 in a bankcard. This additional compensation will be an option for the 4 study visits.

10 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).

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