Dyslipidemia of Obesity Intervention in Teens
DO IT! Trial

PROTOCOL

VERSION 6.0
Date: July 1, 2019

Sponsor:
The National Heart, Lung, and Blood Institute (NHLBI), NIH/DHHS

Data Coordinating Center (DCC):
New England Research Institutes, Inc.

APPROVALS:

Sponsor: Gail Pearson, MD, ScD

Signature: _____ Date: _____

DCC: Julie Miller, MPH, PMP

Signature: _____ Date: _____

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1. GENERAL INFORMATION

1.1 Version History Summary

Version Number	Version Date	Comment(s)
Version 1.0	September 8, 2014	Initial version sent to the FDA.
Version 2.0	May 18, 2017	Incoporated recommendations from the FDA on exclusion criteria, updated vascular measures procedures and blood draw amounts.
Version 3.0	June 20, 2017	Lengthened recruitment period, included lifestyle measurements, streamlined schedule of measurements
Version 4.0	September 25, 2017	Clarified exclusion and drug discontinuation criteria, added sleep quality measurements, removed unnecessary measurements, included new package insert information.
Version 4.1	January 18, 2018	Incorporated recommendations from Health Canada per their regulation guidelines.
Version 5.0	January 4, 2019	Revised eligibility criteria, lengthened recruitment period, clarified screening visit procedures and schedule of visits, clarified reasons for study drug discontinuation.
Version 6.0	July 1, 2019	Elimination of visits at 3 and 9 months. New package insert from drug manufacturer.

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1.2 Protocol Signature Page

I have read the foregoing protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study in accordance with the design and specific provisions outlined herein; deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the drug and the conduct of the study.

I will use the informed consent form approved by the NHLBI and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board or Ethics Committee responsible for this study.

I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse experiences as defined in **Section 7** of this protocol.

I further agree that the NHLBI and/or its designee has access to any source documents from which case report form information may have been generated.

I also agree to handle all clinical supplies (including drugs, biologics, and/or devices) provided by vendors and collect and handle all clinical specimens in accordance with the protocol.

The below signed confirm herewith to have read and understood this study protocol and/or amendment and appendices; furthermore, to accomplish this study in accordance to the protocol and Good Clinical Practice guidelines, as well as local regulations and regulatory authorities.

PRINTED OR TYPED NAME(S)	SIGNATURE	DATE
Investigator		
Investigator		
Investigator		
Investigator		

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1.3 Protocol Synopsis

Title	**Dyslipidemia of Obesity Intervention in Teens – DO IT! Trial**
Grant Number	NHLBIU01 HL068270, HL109741, HL109781, HL109816, HL109818, HL109777, HL109778, HL109673, HL109743, HL109737
Study Objectives	To determine if treatment of combined dyslipidemia of obesity (CDO) in adolescents with oral pitavastatin will improve vascular measures of early atherosclerosis with acceptable safety.
Significance	Combined dyslipidemia (CDO) is prevalent among obese adolescents and is highly atherogenic, making it an important public health problem. While lifestyle interventions aimed at reducing adiposity are being pursued, additional effective treatment of the dyslipidemia is indicated to halt or slow the progression of atherosclerosis. This trial of pitavastatin will determine efficacy and safety in this high risk population and provide evidence for clinicians to target this treatable risk factor to achieve an impact on early atherosclerosis, and potentially achieve primary prevention of adult cardiovascular disease.
Study Design	Randomized, double-blind, placebo-controlled clinical trial of pitavastatin for 2 years.
Primary Aim	To compare the effect of pitavastatin versus placebo on vascular measures in at least 354 adolescents with excess adiposity (body mass index [BMI] ≥85th percentile) and CDO (defined as high non-HDL-C + high TG/HDL-C ratio or low HDL-C).
Secondary Aim(s)	1. To compare the effect of pitavastatin versus placebo on safety measures including adverse events. 2. To compare the effect of pitavastatin versus placebo on lipid profile measures.
Accrual Objective	At least n=354 randomized (177 per group).
Study Duration	Recruitment will occur over an estimated period of 3 years. The duration of observation for each study participant will be 2 years.

Inclusion Criteria	• Boys and girls aged 10 to 18 years (with 2 year availability for study participation)
	• BMI ≥85th percentile (using CDC BMI charts)
	• Fasting lipid profile x2 each with all of the following:
	  ○ LDL-C <160 mg/dL and ≥90 mg/dL, and
	  ○ TG <500 mg/dL, and
	  ○ TG/HDL-C ratio ≥2.5 or HDL-C <45 mg/dL for boys or HDL-C <50 mg/dL for girls, and
	  ○ non-HDL-C ≥125 mg/dL
	• Participant consent, or parental/guardian consent and participant assent

Exclusion Criteria	• Current use of lipid lowering medication, antihypertensive medication, growth hormone, systemic corticosteroids,

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	cyclosporine, protease inhibitors, erythromycin, rifampin, colchicine, warfarin, second generation psychotropic drugs, oral isotretinoin; stable doses of stimulant or antidepressant therapy will be accepted • Known allergy or hypersensitivity to statin • Patients who have had bariatric surgery or plan to have bariatric surgery during the trial • Female who is pregnant, plans to become pregnant or is sexually active without contraception • Stage 2 hypertension (systolic or diastolic blood pressure ≥95th percentile for age, sex and height percentile + 12 mmHg or ≥140/90, whichever is lower for participants <13 years of age; ≥140/90 for participants ≥13 years of age) confirmed after an appropriate evaluation) • Diabetes (type 1 or type 2) by American Diabetes Association criteria (fasting glucose ≥126 mg/dL, HbA1c ≥6.5%, random glucose ≥200 mg/dL, or 2-hour oral glucose tolerance testing glucose ≥200 mg/dL) • Use of insulin sensitizing therapy • Known renal insufficiency (known chronic renal disease, estimated GFR <60 mL/min/1.73m^2 at screening) • Uncontrolled thyroid disease (TSH at screening >1.5x upper limit of normal, clinical or other laboratory evidence of hypothyroidism, or thyroid hormone therapy that has not been stable for 6 weeks prior to screening) • Proteinuria suggestive of renal disease (more than trace together with an elevated urine protein:creatinine ratio as per local lab) • Syndromic patients or patients with neurocognitive delay precluding adherence with study drug • Liver disease other than non-alcoholic fatty liver disease (NAFLD) either diagnosed or suggested by alanine amiotransferase (ALT) ≥ 40 U/L, or severe NAFLD indicated by ALT ≥ 200 U/L • Unexplained persistent elevated creatine kinase (CK) level >3x upper limit of normal • Plans to leave the geographic area before completion of the anticipated 2 years of trial participation • Any unstable medical or emotional condition or chronic disease that would preclude following the protocol or impact valid vascular measurement • Admits to current smoking, current alcohol consumption

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1.5 List of Abbreviations

ADA	American Diabetes Association
AE	Adverse Event
ALT	Alanine Aminotransferase
Apo AI	Apolipoprotein AI
Apo B	Apolipoprotein B
Apo CIII	Apolipoprotein CIII
ARIC	Atherosclerosis Risk In Communities Study
AST	Aspartate Aminotransferase
Beta	Stiffness Index Beta
BMI	Body Mass Index
BP	Blood Pressure
CABG	Coronary Artery Bypass Grafting
CBC	Complete Blood Count
CDO	Combined Dyslipidemia of Obesity
CDC	Centers for Disease Control
CIMT	Carotid Intima Media Thickness
CK	Creatine Kinase
cm	Centimeter
CRF	Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
CVD	Cardiovascular Disease
CYP3A4	Cytochrome P450 3A4
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMS	Data Management System
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
Einc	Incremental Elastic Modulus
FDA	Food and Drug Administration
FH	Familial Hypercholesterolemia
FLP	Fasting Lipid Profile
GI	Gastroenterology, Gastroenterologist
HbA1c	Hemoglobin A1c (glycosylated hemoglobin)
HDL	High Density Lipoprotein
HDL-C	High Density Lipoprotein - Cholesterol
HDL-P	High Density Lipoprotein - Particle
HIPAA	Health Insurance Portability and Accountability Act
HOMA	Homeostasis Model of Assessment – Insulin Resistance
HR-QOL	Health-Related Quality of Life
hs-CRP	High Sensitivity C-Reactive Protein
HTN	Hypertension
Hx	Medical History
IL-6	Interleukin-6

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IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	Intention to Treat
kg/m^2	Kilograms per Meter Squared
LDL	Low Density Lipoprotein
LDL-C	Low Density Lipoprotein - Cholesterol
LDL-P	Low Density Lipoprotein – Particle Number
Lp(a)	Lipoprotein (a)
m/sec	Meters per Second
mg/dL	Milligrams per Deciliter
MM	Medical Monitor
mm	Millimeters
NAFLD	Non-Alcoholic Fatty Liver Disease
NDSR	Nutritional Data System for Research
NHANES	National Health and Nutrition Examination Survey
NHLBI	National Heart, Lung and Blood Institute
NIH	National Institutes of Health
NITT	Non-intention to Treat
NMR	Nuclear Magnetic Resonance
Non-HDL-C	Non-High Density Lipoprotein - Cholesterol
OGTT	Oral Glucose Tolerance Test
PAI-1	Plasminogen Activator Inhibitor-1
PCOS	Polycystic Ovarian Syndrome
P/E	Physical Examination
PEM	Peterson Mean Pressure-Strain Elastic Modulus
PHN	Pediatric Heart Network
PI	Principal Investigator
PWV	Pulse Wave Velocity
QA	Quality Assurance
QC	Quality Control
QUICKI	Quantitative Insulin Sensitivity Check Index
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SES	Socioeconomic Status
SID	Study Identification Number
sRBP-4	Serum Retinol Binding Protein-4
SV	Screening Visit
TG	Triglycerides
TNF-α	Tumor Necrosis Factor - alpha
TSH	Thyroid Stimulating Hormone
U/L	Units per Liter
ULN	Upper Limit of Normal
VLDL-C	Very Low Density Lipoprotein - Cholesterol
WHO	World Health Organization
YEM	Young’s Elastic Modulus

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2. STUDY AIMS AND HYPOTHESES

2.1 Primary Aim

Primary Aim: To compare the effect of pitavastatin versus placebo on vascular measures in at least 354 adolescents with excess adiposity (body mass index [BMI] ≥85th percentile) and combined dyslipidemia of obesity ([CDO] defined as high non-HDL-C + high TG/HDL-C ratio or low HDL-C).

Primary Hypothesis: Over 2 years of observation, participants in the pitavastatin group will have a significantly different change over time (slope) in pulse wave velocity (PWV) than those in the placebo group.

Study Outcomes: Pulse wave velocity (PWV); secondary measures include carotid intima media thickness (CIMT) and carotid artery stiffness.

2.2 Secondary Aims

Secondary Aim 1: To compare the effect of pitavastatin versus placebo on adverse events.

Hypothesis: Participants in the pitavastatin group will demonstrate no excess clinically relevant laboratory abnormalities or symptoms, and will have normal growth compared to those in the placebo group.

Study Outcomes: Liver function tests (ALT, AST); creatine kinase (CK), muscle symptoms; markers of glycemic control/development of diabetes (fasting plasma glucose, HgbA1c) and change in surrogate markers of insulin sensitivity (fasting insulin, C-peptide, HOMA-IR, 1/insulin, QUICKI); height velocity (change in height z score); prevalence of adverse events and other participant-reported symptoms (including neurocognitive and depressive symptoms).

Secondary Aim 2: To compare the effect of pitavastatin versus placebo on lipid measures.

Hypothesis: Participants in the pitavastatin group will demonstrate significantly greater reductions in non-HDL-C, TG/HDL-C ratio, LDL-C, LDL particle number (LDL-P) than participants in the placebo group. Participants in the pitavastatin group will demonstrate significantly greater increases in HDL-C and HDL particle number (HDL-P) than participants in the placebo group.

Study Outcomes: Standard fasting lipid profile and apolipoproteins; NMR spectroscopy for lipoprotein particle characterization and quantification.

3. BACKGROUND INFORMATION

3.1 Background on Condition, Disease, or Other Primary Study Focus

Adiposity is a critical public health problem. Obesity is prevalent, affecting 16.9% of American children and adolescents,[1,2] and is associated with increased short-term and lifetime healthcare expenditures compared to normal weight children.[3] Three quarters of overweight adolescents become obese adults,[4,5] which has important implications for future population health.[6,7] Long term sequelae of childhood obesity include metabolic syndrome, type 2 diabetes mellitus, premature cardiovascular disease (CVD) and early mortality.[8-11] In the short term, 50% of obese adolescents have at least one, and 10% have 3 or more CVD risk factors, including hypertension, dyslipidemia, and insulin resistance.[12,13] The prevalence of metabolic risk factors increases with increasing severity of obesity.[14] Lipid abnormalities are strikingly common: 39% of obese youth have some abnormal lipid value,[15] typically high TG and low HDL-C, which are components of CDO.[16] With the increased prevalence of pediatric obesity, secondary causes of dyslipidemia have superseded primary etiologies of cardiovascular risk in youth.[17]

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Adiposity leads to a highly atherogenic lipid phenotype. In the obese, insulin resistant individual, increased free fatty acid levels stimulate overproduction of TG-rich lipoprotein particles in the liver, primarily VLDL, clinically manifested as high TG.[18] Insulin resistance promotes lipoprotein lipase dysfunction, further elevating TGs. Insulin is secreted at high levels to accommodate excess glycemic calories and to overcome resistance at the tissue level. Transfer between LDL and HDL particles of TG in exchange for cholesterol leads to smaller, denser and more atherogenic particles.[19] Small LDL particles are less readily cleared, leading to elevation of total LDL particles[20] and increased risk of their entrapment and retention in the arteriolar subendothelial matrix.[21] Small HDL particles are conversely more rapidly catabolized, contributing to a reduction in reverse cholesterol transport and lowered HDL-C. These factors combine to produce CDO, seen on a standard lipid profile as high non-HDL-C and TG and low HDL-C, and on nuclear magnetic resonance (NMR) spectroscopy as increased LDL particle number (LDL-P), mostly from small, dense LDL particles, and reduced large HDL particles (HDL-P).[22] Non-HDL-C is a strong correlate for total LDL particle number, while the TG/HDL-C ratio is the stronger correlate of small,dense LDL particles.[23,24]

CDO is strongly associated with other cardiometabolic risk factors.
Several studies show CDO is associated with insulin resistance, elevated BMI and central adiposity. The HEALTHY study characterized lipids in a diverse population of 2384 sixth grade children and found that 33% of overweight/obese children had a TG/HDL-C ratio >3.0 and 11.2% had non-HDL-C >145 mg/dL.[25] These findings are consistent with a hyperinsulinemic–euglycemic clamp study that showed that CDO (expressed as elevated TG/HDL-C ratio) was associated with *in vivo* insulin resistance,[26] and with NHANES data that showed that elevated non-HDL-C was associated with the metabolic syndrome in childhood.[27] The relationship between TG/HDL-C ratio and non-HDL-C, and NMR spectroscopy data on small, dense and total LDL particles, has been confirmed in pediatrics in a high risk clinic population,[23] and in the larger school-based HEALTHY study.[24] Of note, TG/HDL-C and non-HDL-C cutpoints used to identify adverse LDL-P and smaller LDL size differ by race/ethnicity in obese adolescents. Small and total LDL-P levels were higher in whites with TG/HDL-C ratio ≥ 3 and non-HDL-C ≥120 mg/dL; equivalent cutpoints for black obese adolescents were TG/HDL-C ratio ≥ 2.5 and non-HDL ≥145 mg/dL in a clinic population.[23] TG/HDL-C ratio ≥ 3 and non-HDL-C ≥ 145 mg/dL identified the presence of CDO comparably in the HEALTHY study.[24] As noted in both studies, CDO was identified with good sensitivity and specificity by elevated TG/HDL-C ratio and non-HDL-C. CDO was noted to be prevalent and related to multiple indicators of adiposopathy. Specific to our inclusion criteria for the DO IT trial, both HDL-C and large HDL particles were significantly lower, and small HDL particles and small LDL particles were higher (but notably not total cholesterol and LDL-C), in the subset of dyslipidemic middle school youth with central obesity and insulin resistance as assessed by waist circumference and HOMA-IR.

Combined dyslipidemia is strongly related to adult CVD and atherosclerosis markers in children. While CV disease rates are falling in the larger population in parallel with declines in tobacco use, total cholesterol and improved blood pressure control, this decline is not occurring in obese adults, particularly women, below the age of 55 years.[28] This is largely secondary to the comorbidities of excess central weight and insulin resistance, including CDO and the downstream development of type 2 diabetes. CDO is the most common lipid pattern seen with clinical CVD. In the Framingham Offspring Study, CDO on standard lipid profile presages early clinical CVD events[29] and identifies the presence of elevated small LDL-P with advanced lipid testing, which has also been shown to predict CVD events.[30] In a long-term pediatric cohort study, the Princeton Follow-up Study, CDO (elevated TG and TG/HDL-C ratio, low HDL-C) at 12 years of age predicted clinical CVD events at late follow-up 3 to 4 decades later.[31] **This is the first childhood lipid parameter shown to be associated with clinical CVD events**.

Evidence from autopsy studies and subclinical vascular testing has defined the strong relationship between CDO and accelerated atherosclerosis. In both the Pathobiological Determinants of Atherosclerosis in Youth Study and the Bogalusa Heart Study, high non-HDL-C and low HDL-C were strongly associated with autopsy evidence of premature atherosclerosis.[32,33,34] Subclinical vascular testing has also been shown to be related to CDO. In adults, low HDL-C and high TG independently predict higher carotid artery intima media thickness (CIMT),[35] and higher TG/HDL ratio predicts CIMT progression.[36] Risk factors measured in young adulthood (18-30 years of age) are better predictors of future coronary artery calcium (a subclinical atherosclerosis measure associated with risk reclassification in adults) than risk measured at the time of coronary artery calcium assessment.[37,38] Adults with CDO have been shown to have higher pulse wave velocity (PWV),[39, 40] and higher baseline non-HDL-C levels lead to higher PWV at follow-up.[41] The Atherosclerosis Risk in Communities (ARIC) study found that incident stroke was associated with higher/worse carotid artery stiffness.[42] Higher PWV was an independent predictor of myocardial infarction in the Framingham Heart Study.[43] CDO identified in childhood has been shown to be associated with vascular damage measured in adulthood by CIMT and PWV.[41,44] Even in childhood, CDO is related to subclinical vascular findings. High TG[45, 46] and low HDL[47] in youth were independent predictors of increased CIMT, especially in those with full metabolic syndrome.[48] Obese youth with elevation in TG and low HDL-C have been shown to have thicker CIMT, higher PWV and increased carotid artery stiffness.[49-51] A strong association between higher TG/HDL-C ratio, higher non-HDL-C and higher PWV in both lean and obese children has been reported, even after adjusting for other CVD risk factors.[52]

3.2 Prior Studies

Lifestyle change interventions improve combined dyslipidemia, but residual risk remains. Weight loss,[53-57] isolated changes in dietary composition,[58-63] and changes in physical activity[64-73] have all been shown to improve CDO in adults and children in short term studies. These lifestyle change interventions significantly reduce TG, non-HDL-C and TG/HDL-C ratio, and lead to LDL-P that is larger and, therefore, more readily cleared, as noted in both adults and children.[53, 55-62,68-74,75,76] Refined carbohydrate restriction shows particular promise for decreasing TG levels in adults[58] and children.[57,59-63] A fiber-rich nutrient-dense supplement has been shown to significantly increase total HDL-C and large HDL particles,[77] an outcome that is delayed in the overweight and obese youth with chronic inflammation.[78] Interventions based on increased activity alone or that combined weight loss with activity showed significant increases in HDL-C.[53,56,66,72,79-81] Although these results are encouraging, the lifestyle modification interventions so far reported have not been sufficient to address the long term cardiovascular risk of CDO. For example, a pilot study was performed in obese white youth with CDO of a 6-month clinic-based intervention that limited refined carbohydrates and promoted 30 minutes of activity per day.[82] Participants achieved a 0.4 kg/m^2 decrease in BMI, an approximate 50% decrease in TG and an approximate 20 mg/dL decrease of non-HDL-C. Despite these improvements, post-intervention lipid levels were still elevated in the range associated with increased cardiometabolic risk, with average TG levels of 196±111 mg/dL and non-HDL-C of 151±47 mg/dL.

Statins effectively treat CDO in adults; their efficacy in obese children and adolescents is unknown. A characteristic finding of CDO is the proliferation of small LDL subspecies, which are cleared less efficiently by LDL receptors. In adults with CDO, statin therapy has been shown to beneficially alter both the standard lipid and LDL particle profiles, by ~40-50%.[83-90] Statins lead to an increase in LDL receptors, as demonstrated in children[91] and adults,[92] and should be synergistic with lifestyle management. Statins are also known to be anti-inflammatory, which may be important in the pro-inflammatory state of adiposopathy.[93] The safety and LDL-lowering

effects of statins has been shown in several high-risk pediatric populations including type 1 diabetes mellitus,[94] dyslipidemia in renal insufficiency,[95] renal transplantation,[96,97] heart transplantation,[98] and systemic lupus erythematosus.[99] However, no evidence exists regarding the safety and efficacy of pharmacologic treatment for CDO in childhood, for any statin.

Statins and lifestyle modification improve subclinical vascular abnormalities, surrogate indicators of CVD. Epidemiologic studies in adults have used hard CV events as the endpoint evaluation of treatments for dyslipidemia.[100] However, subclinical vascular measures have been used as surrogate measures for CVD events in trials when the latency between exposures (adverse lipid profiles) and outcomes (CVD events) is decades long, and using clinical events as outcomes is untenable. The usefulness of these measures is shown by the large body of evidence demonstrating the strong link between measures of arterial stiffness such as PWV and hard CV events in high risk participants with kidney disease,[101,102] diabetes,[103] and hypertension.[104,105] Of more relevance, however, are data from longitudinal cohorts of 'healthy adults' such as the Hoorn[106] and Framingham studies[43] which show that PWV is an independent predictor of CV events even after correcting for current levels of CV risk factors. This association is likely due to the ability of PWV to reflect atherosclerotic burden as seen in studies relating PWV to coronary calcium score,[107] coronary plaque,[108] and coronary ischemia on exercise tests.[109] Similarly, carotid stiffness predicts CV outcomes[110-113] with the strongest evidence from large studies such as the ARIC study[42] and a recent meta-analysis that included over 22,000 participants.[114] CIMT has also been shown to add to traditional risk algorithms in prediction of CVD in studies of over 38,000 participants from the ARIC,[115,116] Rotterdam,[117,118] Tromso,[119,120] CV Health studies[121,122] and Multi Ethnic Study of Atherosclerosis (MESA).[123] Furthermore, progression of CIMT predicts vascular events[124] and TG and TG/HDL-C ratio may be the strongest predictors of CIMT progression.[36] Better vascular health in adults can be related to healthy childhood risk status and behaviors, as shown in the Bogalusa Heart Study,[125] and in the Cardiovascular Risk in Young Finns study, where sustained low-risk status and healthy diet from childhood to adulthood was associated with thinner CIMT.[126,127] In adults, a small number of studies have shown that lifestyle interventions improve subclinical vascular measures, specifically PWV.[128,129] Interventional studies to improve vascular health in youth are limited; one small study of a one-year weight loss intervention in pre-pubertal children found that those participants who were successful in weight loss had a decrease in CIMT.[130] More data are available about the effect of statins on subclinical vascular measures. In adult trials, statin treatment has been shown to be associated with reduced CV events and atherosclerosis regression on CIMT and intracoronary ultrasound.[131,73,74,132,133] Statins were noted to improve arterial stiffness in three recent adult studies, including one using low-dose rosuvastatin,[134] one using fluvastatin,[135] and a study of pitavastatin (the statin to be used in this trial).[136]

There have been few pediatric trials of statin treatment that assessed impact on vascular measures. Treatment of children with familial hypercholesterolemia (FH), who have predominantly LDL-C elevations, with simvastatin normalized endothelial function compared to normal control participants.[137] Another trial of children with FH showed regression of CIMT for those treated with pravastatin, while those who received placebo showed the expected age-related progression.[138] Patients from this trial have now been followed for 10 years, and treated patients have shown a similar age-related progression of CIMT compared to that of their unaffected siblings, with no increase in adverse events.[139] One small 12 week cross-over trial of 20 mg of atorvastatin in 50 children with type 1 diabetes suggested reduced arterial stiffness using radial artery tonometry measured with the same device proposed for this study.[140] **No published studies have examined statin effects on vascular health (PWV or CIMT) in adolescents with CDO.** This study will address these knowledge gaps.

3.3 Rationale for the Study

The rationale for targeting this specific high risk dyslipidemic population is detailed in the background and rationale sections of the protocol, and is summarized as follows:

a) The prevalence of CVD is not falling in adults <55 years of age, particularly women.[28] This is most likely explained by obesity and associated co-morbidities, particularly combined dyslipidemia, hypertension and type 2 diabetes.
b) The prevalence of excess adiposity is now higher in adolescents than for the generation captured in the above analyses; thus, the related morbidity will likely increase. Outcomes from studies such as the Princeton High School study have linked obesity-related combined dyslipidemia in youth to future diabetes and CVD. From the autopsy studies, as well as studies tracking vascular markers of subclinical atherosclerosis, the cohort recruited into the proposed trial likely has a similar atherosclerosis burden and progression to those with mild FH already approved for treatment.
c) Lipid-lowering trials are traditionally done in individuals >50 years of age and often in high risk patients so that the CVD event rates are sufficiently high to complete a trial in 3-5 years. The longer term downstream benefits of these interventions are rarely studied.
d) Statin treatment may be more effective earlier in the course of atherosclerosis. Trials in adults have demonstrated a legacy effect, whereby participants in the intensive treatment arm continue to have lower event rates than the less intensive treatment group years after conclusion of the trial. Further, the biggest relative risk reduction observed in statin trials is in those with the lowest Framingham risk score, which is largely driven by younger age.[141]
e) Many of the adolescents eligible for the proposed trial, if unrecognized and untreated, would be expected to have had events by the time they would be old enough to enter most statin trials in adults upon which current evidence is derived. This creates a bias against younger individuals with high risk who have the greatest need for prevention.
f) Reliance on hard CVD events as the only valid trial endpoint makes trials of true prevention impossible to conduct.[142] Studying the impact of therapies on CVD outcomes in youth is not feasible, nor is an ultra-long-term study reasonable. However, existing models predict that treating youth early will achieve greater benefits downstream. Improvement in surrogate markers of subclinical atherosclerosis becomes important to demonstrate that the intervention has an impact on atherosclerosis. The current paradigm is shifting to preventing atherosclerosis rather than just preventing CVD events.[141] The proposed trial aligns with this change in thinking.
g) Other evidence that the benefits from early treatment may be greater than later treatment includes intravascular ultrasound studies from statin trials in adults. These have demonstrated an impact on increasing plaque stabilization by increasing plaque fibrosis, but minimal impact on lipid or necrotic cores in lesions.[143,144] Residual risk in these trials is likely explained by this fact, and only earlier treatment can prevent development of advanced atherosclerosis. Studies in FH comparing affected children to their affected parents show an impact on events of statin treatment early in life.[145, 146]
h) The safety profile of statins may be more favorable with early treatment. Dyslipidemic obese adolescents will have higher likelihood of developing diabetes than other pediatric cohorts, and also are at risk for fatty liver disease; therefore, defining safety

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outcomes is critical. Adult studies do not have these data in 20-50 year olds, hence, a pediatric trial is necessary.

i) The proposed trial will enroll a cohort for whom the 2011 Expert Panel guidelines would consider therapy.[147]

Statins have been effective in treating CDO in adults, with beneficial effects on vascular measures. Pediatric statin trials of children and adolescents with FH have shown excellent lipid-lowering and safety, similar to findings from trials in adults. Specifically, pitavastatin has been studied as an LDL lowering agent in children with severe dyslipidemia. A small double blind placebo controlled study conducted in 14 Japanese boys showed good LDL-C lowering and a good safety profile.[148] A second pediatric trial involved 10 centers from 6 European countries (the PASCAL Study).[149] Participants ages 6 to 17 years, including some children with LDL-C ≥130 mg/dL with additional risk factors, but with the majority having FH (mean baseline LDL-C 234 ± 52 mg/dL), were randomized to a 12-week double-blinded study of 1, 2, or 4 mg versus placebo (n=106), with an open label extension during which most were up titrated to 4 mg daily (n=113). Children experienced an LDL-C reduction of 23.5% with 1 mg, 30.1% with 2 mg, and 39.3% with 4 mg of pitavastatin. Similar reductions were seen in non-HDL-C and apoB, with no change in HDL-C or triglycerides. There were no significant increases in AST, ALT and CK, and no differences in adverse events.

Effective treatment of CDO in youth with a statin while pursuing efforts at reducing adiposity is justified, given the compelling evidence of the presence of accelerated atherosclerosis and the lack of sufficient efficacy of lifestyle interventions. Some obesity-related comorbidities share similarities with adverse effects reported for statins. These include a possible increased risk of new onset diabetes associated with statin use versus the increased risk of type 2 diabetes associated with obesity, and the risk of increased hepatic transaminases associated with statin versus progression of non-alcoholic fatty liver disease associated with obesity. These unknown but important safety considerations further justify rigorous research methodology. This clinical trial of statin for treatment of CDO in obese youth will be the first pediatric trial to study statin therapy outside of the setting of FH, and will provide crucial evidence to guide clinical decision-making in a population at very high risk for premature CVD.

3.4 Rationale for the Study Outcomes

3.4.1 Vascular Outcomes.

CDO in adults is also associated with subclinical vascular disease including increased CIMT[35] and higher PWV, which are the vascular measures to be used for this trial.[39,150] CDO is reflective of LDL particle infiltration and may be the etiology for this accelerated atherosclerosis, since increased TG/HDL-C ratio[151] and small dense LDL predict greater CIMT independent of other CVD risk factors.[152,153] Of greater importance is the observation that CDO identified in childhood is associated with increased CIMT[48,154-158] and PWV[41] in adults. These data suggest an increased risk for future CVD events in youth, since CDO leads to carotid[159] and PWV[43] abnormalities which predict hard CVD events in adults.[124,160,161] Preliminary work has found that thicker CIMT,[49] and higher PWV[50,162] in adolescents are associated with obesity-related dyslipidemias. A recent study demonstrated a strong association between TG/HDL-C ratio and higher PWV in obese adolescents. Importantly, TG/HDL-C ratio was an independent predictor of PWV, even after adjusting for other CVD risk factors.[52] In summary, obesity leads to CDO-related increases in small, dense LDL, which leads to atherosclerosis-related vascular damage that increases risk for adult CVD.

PWV was chosen as the primary outcome measure over CIMT as its measurement is more reproducible when applied across multiple study sites. In addition, preliminary data support both associations with CDO in adolescents, and responsiveness to statin in short-term trials in adults, together with its relationship to CVD in adults. Statins improve PWV, as demonstrated by multiple adult studies,[134,135,163] including some that showed improvement in PWV in as few as 4 to 12 weeks.[164-167] It is also easier to assess, is more reproducible and is easier to standardize across multiple sites than other non-invasive sub-clinical atherosclerosis measures such as brachial artery reactivity.

CIMT will be measured as a second important primary outcome, since it is a measure of vascular structure. It is linked to hard CVD events in adults and improves risk prediction above and beyond that provided by traditional CVD risk factors such as obesity, diabetes, and hypertension.[160,161] CIMT has been directly correlated to the extent of atherosclerosis on autopsy.[168] Furthermore, atherosclerosis develops in a non-uniform fashion throughout the arterial tree[169] and combining structure (CIMT) and functional changes (PWV, carotid artery stiffness) gives a better representation of overall atherosclerotic burden. In adults, the proportion of small dense LDL particles and changes in this proportion are predictive of changes in both CIMT and insulin resistance.[170] Statin treatment leads to regression of CIMT in adults.[132,133] Recent studies with pitavastatin have shown improvements in CIMT[171] and carotid arterial stiffness, in addition to improvements in endothelial function.[136] Additionally, statin administration has been shown to induce regression of CIMT in youth with FH,[138] with 10 year follow-up data showing the same rate of progression as unaffected siblings.[139] Importantly, the same group demonstrated that age at initiation of statin treatment was an independent predictor of final CIMT (adjusted for baseline value);[172] additional justification for a statin trial in youth with CDO. No data are available on the effect of statins on CIMT or carotid artery stiffness in youth with CDO. The rationale for using CIMT as the second primary outcome is that regression of CIMT is a slower process requiring a minimum of 2 years of treatment; and the proportion of the variance in CIMT explained by traditional CVD risk factors is lower than for PWV,[173] suggesting that other genetic and environmental factors may play a role.

Carotid stiffness measures also predict CV events in adults, including CV mortality[110] and stroke.[174] Studies in youth demonstrate a relationship between carotid stiffness and CV risk factors including environmental tobacco exposure,[175,176] obesity,[49,177] metabolic syndrome-like risk factor clustering,[178,179] hypertension,[162] and type 2 diabetes.[49] Stiffer carotids are also found in adolescents with type 1 diabetes[180-182] and chronic kidney disease.[183] A higher incremental elastic modulus in youth has been shown to be associated with a higher TG/HDL-C ratio.[52] No studies of statins to improve carotid stiffness in youth have been conducted to date, but adult studies demonstrate the effectiveness of this approach.[167]

3.4.2 Safety

Safety outcomes for the trial are based on known statin side effects and special considerations related to adolescents. Safety outcomes are crucial in a pediatric statin trial. The major safety side effects considered for this trial are muscle toxicity, liver toxicity, and incident diabetes. Additionally, other patient-reported subjective symptoms (including neurocognitive and depressive symptoms) will also be serially collected throughout the trial. Published randomized trials of statins in children and adolescents with FH have not reported significant toxicity or side effects, nor impact on growth and sexual maturation.[184,185]

- Muscle toxicity: Muscle toxicity has been reported in 1% to 28% of participants in adult statin trials, with the incidence varying by type of study design (lower incidence in randomized trials and higher in observational studies) and study exclusion criteria (lower

if patients likely to have muscle issues are excluded).[186] A recent rigorous randomized trial in adults noted that muscle pain was slightly more frequent in adults taking statins but symptoms were not associated with alterations in creatine kinase (CK) levels or exercise performance.[187,188] Cases of severe rhabdomyolysis are exceedingly rare in adults[189] and unreported in children or adolescents. Pediatric trials and clinical experience do not suggest muscle toxicity occurs frequently in children.[184] A definition of muscle toxicity adapted from standard adult definitions has been adopted for this trial.[186] (see **Sections 4.3.2, 5.3.1** and **6.2.4**)

- Liver toxicity: Combined dyslipidemia is also strongly linked with non-alcoholic fatty liver disease (NAFLD), defined as hepatic fat infiltration in >5% of hepatocytes with no evidence of hepatocellular injury on liver biopsy and no history of alcohol intake.[190] NAFLD is highly correlated with obesity, affecting at least 38% of obese adolescents in autopsy series and ~50% in epidemiologic surveys.[191,192] On evaluation, the most common findings are hepatomegaly and mild-to-moderate elevation in serum alanine aminotransferase (ALT).[190,193-195] Hepatic fat deposition usually occurs in the context of generalized obesity, but reflects much more strongly the presence of increased visceral adiposity. In obese children and adolescents, sequential increase in waist circumference, a proxy measure of visceral fat, is associated with progressive increase in odds ratio for prediction of ultrasound-detected hepatic steatosis.[195] NAFLD is strongly associated with insulin resistance and all of the components of the metabolic syndrome.[195-197] In a study of adolescents with biopsy-proven NAFLD, 80% had biochemical evidence of insulin resistance.[196] In more than half of participants with NAFLD, the atherogenic combined dyslipidemia pattern is seen on a standard lipid profile and with NMR analysis.[197] In children and adolescents, NAFLD is associated with atherosclerosis at autopsy and with ultrasound vascular markers associated with atherosclerosis.[198] In adults, NAFLD has been shown to be a strong, independent predictor of CVD.[199]

  Review of the statin treatment literature for children and adults, including recent trials of pitavastatin, does not support the presence of significant liver toxicity from statins. High dose statins may increase levels of hepatic transaminases in older individuals, but these high doses are not proposed for this trial.[184,200,201] However, it is expected that this cohort of obese adolescents will have a high prevalence of NAFLD, and participants may have associated elevated ALT levels.[202] Thus, assuring safety of statins in this population is important. Statins have been studied both in the context of NAFLD and as a treatment for NAFLD.[203,204] Treatment of dyslipidemia in the setting of NAFLD is recommended in adults, and may improve liver function tests. A pilot study of pitavastatin in adults with NAFLD showed improved liver histology in about half of the participants when treated for 12 months.[205,206] Monitoring of liver function is included in the protocol and worsening of liver function will be addressed as part of safety monitoring, as per the Table provided in **Section 5.3.1**.

- Diabetes: In adult studies, statin therapy increases the risk of new-onset diabetes.[207] This risk is related to the intensity of therapy, the statin used and degree of LDL-C lowering.[208-210] The mechanism(s) are postulated to be through impaired beta cell function and/or impaired peripheral insulin sensitivity, although a greater understanding is needed in this area. Patients with type 2 diabetes mellitus have been shown to have worse glycemic control on statin. Notably, pitavastatin, the drug chosen for this trial, has not been associated with this effect, which may be mediated by increases in adiponectin.[211] Small studies in adults have shown no effect or improvements in glucose

homeostasis relative to other statins.[212-214] In adult trials, risk/benefit analyses favor continuation of statin treatment in older individuals, as the benefit from reduction of heart disease mortality exceeds morbidity from diabetes. At-risk participants are generally those considered already at risk for type 2 diabetes due to the presence of the metabolic syndrome, which will likely characterize this trial cohort. Pediatric patients enrolled in prior statin trials included only children with FH or severe elevations in LDL-C and, although obese participants were not specifically excluded, there have been no reported effects on fasting glucose and glycosylated hemoglobin (HbA1c), or incident diabetes. A 10 year follow-up study of pravastatin treated FH children showed one incident case of diabetes among 194 patients, compared to one incident case of diabetes among 83 unaffected siblings.[139] Therefore, the trial will carefully monitor diagnostic biomarkers for diabetes, including fasting glucose and HbA1c, in all participants. Further, determination of fasting insulin and C-peptide at the same time points will allow for assessment of surrogate indices of insulin sensitivity[215] and beta cell function.[216] (see **Sections 5.3.1** and **6.2.4**)

3.4.3 Lipid Phenotype

Standard lipid profile measures are highly associated with CDO and identify disproportionate total LDL particle burden and reduced large HDL particle number as described above (**Section 3.1**). Both lifestyle intervention and statin therapy have been associated with beneficial changes in the standard lipid profile and lipid subclass measures.[53,55-62,68,70-72,74-76,84,85,91,92] This was evident in the overall response to intensive lifestyle management in the Diabetes Prevention Trial.[217] Decreases in large, buoyant VLDL particles and small dense LDL particles, and increases in large HDL particles were noted. These beneficial effects were mediated by improvements in insulin sensitivity, weight loss and increased adiponectin. Metformin more modestly decreased small LDL particles. Since TG and HDL-C measurements are easily available to health care providers as part of the standard lipid panel, the use of TG/HDL-C ratio, HDL-C and fasting non-HDL-C as surrogate markers for LDL particle concentration/burden in this trial will have important translational value. This is clinically relevant because total LDL particle number has been shown to be more strongly correlated with CV disease than LDL-C.[30] LDL particle number is not being measured as a criterion for study eligibility, as it is not recommended for standard clinical practice. It must be performed in a specialized lab, data-derived normal cutpoints do not exist, and more routinely available TG/HDL-C ratio, HDL-C and non-HDL-C have been shown to be highly correlated with NMR spectroscopy results.[23] The inclusion of lipoprotein phenotyping as a secondary outcome in the trial will permit validation of cardiovascular risk stratification with the standard lipid profile, and further our understanding of the roles of LDL and HDL particles in vascular change in this high risk population. In a cross sectional study of a population-based Caucasian and Asian cohort, all three PWV measures (peripheral stiffness by brachial-ankle and femoral-ankle and central stiffness by carotid-femoral) were noted to have significant correlations with total and small, dense LDL particle number (all $p<0.0001$) but not LDL-C (all $p>0.1$), independent of race and age.[218] Peripheral measures of vascular stiffness retained a strong relationship to LDL-P in multivariable analyses, but the correlation with central PWV weakened, suggesting that there may be elements of both stiffness and atherosclerosis reflected in the carotid-femoral PWV measure. This trial includes two measures of vascular stiffness (PWV, carotid artery stiffness) and a structural measure of atherosclerosis (CIMT), and the proposed two year intervention is sufficient in length to detect changes in both. Recently, 17 year follow-up in the Malmo Diet and Cancer study[219] showed in 2,679 middle-aged adults that, for both sexes, the identical variables shown in children in the HEALTHY study to predict LDL-P concentration in multivariable analysis[23] (TG, HDL-C, waist circumference, HOMA-IR) also predicted increased carotid-femoral PWV in this study in adults (after adjustment for mean arterial pressure, classical CVD risk factors and drug treatment). In

the JUPITER trial, in the setting of potent statin therapy, HDL particle number was shown to be a better marker of residual risk than HDL-C.[220] The proposed DO IT trial will provide crucial and rigorous evidence to inform an evolving understanding of the role of standard lipid profile measures and LDL and HDL particles in both vascular change and early atherogenesis, immediately applicable to both current clinical care and future research.

There is important overlap in the lipid phenotype between CDO and familial combined dyslipidemias.[221,222] Because of this overlap, the eligibility criteria may permit recruitment of some participants with underlying genetic dyslipidemia.[223] Familial combined dyslipidemias represents a heterogeneous group of disorders that includes familial combined hyperlipidemia, familial dyslipidemic hypertension, hyperapolipoproteinemia B and LDL subclass pattern B. The lipid phenotypes include Friedrickson types IIa, IIb and IV, and the phenotype can be variable within affected family members and over time. No single gene defect has been identified, and the disorder appears to be oligogenic in etiology with expression unmasked or exacerbated by lifestyle factors. The insulin resistance that commonly accompanies obesity is often a factor that drives greater and earlier expression of the phenotype. The prevalence is not well known but estimated at 0.5% to 2.0%. Diagnostic criteria are present for adults, and include an LDL-C >160 mg/dL with TG >200 mg/dL, together with a family history with at least two members affected with a similar lipid pattern, the presence of lipid variability with and between family members, and the presence of premature CVD.[221] The mechanism of increased CVD risk is the presence of increased numbers of apolipoprotein B-containing particles, particularly small, dense LDL-P,[224] which is also the mechanism associated with CDO. While statins do not specifically target small, dense LDL-P, they are associated with reductions in all subfractions of LDL-P.[225] Increased arterial stiffness is evident in patients with familial combined hyperlipidemia.[39] Thus, participants with familial combined dyslipidemia would likewise potentially benefit from statin therapy, and are not excluded in the trial. They may potentially be identified clinically from family history and detailed lipid characterization. In addition to laboratory-based lipid characterization, detailed family history is included, and will be included as a potential covariate in the analysis of outcomes.

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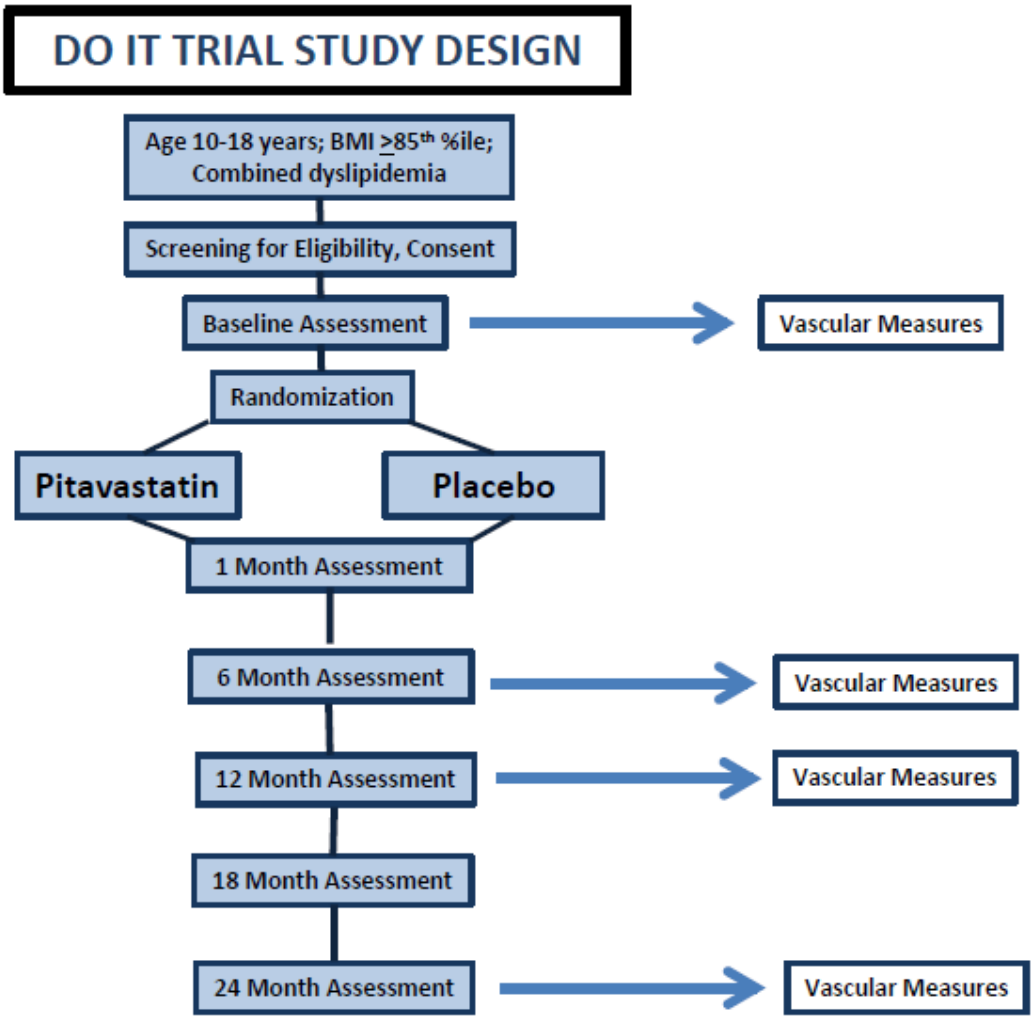
4. STUDY / TRIAL DESIGN

4.1 Overview

The study will be a double-blind 2-arm randomized clinical trial of pitavastatin versus placebo (n=177 randomized in each group) for 2 years (see Figure below).



4.2 Procedures to Minimize/Avoid Bias

4.2.1 Randomization

Participants will be assigned in the order in which they are enrolled into the study to receive an allocated treatment according to a computer-generated randomization plan using the Data Coordinating Center's (DCC) randomization program to generate a treatment assignment code. Randomization will be 1:1 and will be effected using randomly permuted blocks of varying size. Once a participant has been assigned a treatment, the participant will remain on the same treatment for the duration of the study.

4.2.2 Stratification

The randomization scheme will have one stratification factor: clinical center. Randomization will occur within center to ensure that the distributions of allocation of treatment are balanced within a given clinical center.[226] No other stratification criteria will be applied.

4.2.3 Masking

The participant's treatment assignment (pitavastatin versus placebo) will be obtained from the randomization computer at the DCC by the responsible pharmacist. The pitavastatin and placebo pills will be identical in size and shape, ensuring that neither participant nor investigator will be able to determine the treatment assignment. Participants, parents, providers and investigators will be masked to study assignment during the trial. Only designated DCC staff and site research pharmacists will be aware of treatment assignment. Given the relatively low side-effect profile of pitavastatin, it is unlikely, although not impossible, that patients will be able to guess their treatment assignment. All study measures will be obtained without knowledge of the participant's treatment assignment.

Because of the anticipated difference in lipid results between the two groups, knowledge of the full results of fasting lipid profiles would result in unmasking. However, knowledge of TG and HDL-C results are useful in informing lifestyle counseling, and extremely elevated TGs are a safety measure. Knowledge of these results would not be anticipated to result in unmasking, as these values have not been significantly impacted by statins in pediatric trials. For these reasons and for quality control purposes, fasting lipid profiles will be assessed in a centralized core lab, and results of TG and HDL-C only will be shared with the study center team and participants. LDL-C levels will be monitored by the core lab, and if the value is ≥160 mg/dL for more than six months on two successive measures, the DCC and site PI will be informed. This is anticipated to be a rare event, although it may result in a decision to discontinue study drug and start statin therapy based on clinical recommendations (see **Section 6.2.2**).[147] The participant will continue to be enrolled in the trial and have all study measures, as per intention to treat.

Likewise, results from NMR lipoprotein fractions performed in the core lab will not be shared with study sites until all participants at all sites have completed the trial.

4.2.4 Missing Data

In addition to the design features discussed above, careful treatment of missing data as described in **Section 8.5**, below, will help to detect and minimize any possible bias in reporting outcomes of this trial.

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4.3 Study Measures

Vascular	**PWV, CIMT, carotid artery stiffness**
Safety	**ALT, AST; fasting glucose, HbA1c, insulin, C-peptide; muscle symptoms, CK; adverse events**
Lipids	**FLP; LP subfractions (NMR); apolipoproteins**
Clinical and family Hx & P/Exam	**Height, weight, waist circumference; BP; Tanner staging; socioeconomic status; lifestyle assessment**
Lab covariates	**hs-CRP, banked specimens (DNA, plasma/serum)**

4.3.1 Measures of Primary Outcome

Vascular Measures: Pulse Wave Velocity (PWV)

PWV is a functional measure of arterial stiffness associated with atherosclerosis. It is a measure of the time taken by the pressure wave through an artery over a specific distance. PWV is measured from the carotid to femoral arteries and is a reflection of central arterial stiffness along the thoracic and abdominal aorta. Given variation in measurement technique and conditions, normal values do not currently exist.

Method: PWV will be measured with a SphygmoCor device (Atcor Medical, Sydney, Australia).[227] With the patient supine, ECG leads are applied, and the average of 3 measurements of the distance between the two recording sites is obtained (from the carotid pulse above the sternal notch to the femoral artery pulse) and entered into the software. A high-fidelity tonometer (pressure sensor) will be placed on the carotid artery to obtain the 'proximal' pressure waveform and then on the femoral pulse to obtain the 'distal' waveform. The device will calculate PWV as the difference in the carotid-to-distal path length divided by the difference in R-wave-to-waveform times (Δdistance/Δtime, m/sec). PWV will be measured at baseline, and at 6, 12 and 24 months after randomization.

Training: Personnel (technicians, research assistants or nurses) from all sites will attend a PWV training webinar conducted by the vascular core lab. Following this, each observer will perform replicate PWV studies on volunteers; these studies will be transmitted to the core lab for analysis where reproducibility will be calculated. Observers will be 'certified' if their coefficient of variation for within- and between-visit PWV is < 10%, the threshold deemed acceptable in published guidelines.[50] Sites will be instructed to send 1 practice study every 6 months for review and web-based re-training will be conducted prior to year 2 data collection.

Quality Control (QC): PWV is a very reproducible measure,[228] demonstrating a within-visit coefficient of variation of 6.2% and between-visit coefficient of variation of 5.2% in a recent analyses of adolescents (n>800 of which 2/3 were obese). Every 6 months, reproducibility for within-visit (QC) PWV reproducibility will be calculated as the coefficient of variation among the 3 replicate readings obtained during the visit by the DCC. Re-training via video-conferencing or at in-person Pediatric Heart Network (PHN) Steering Committee meetings will be provided as needed.

Carotid Artery Intima-Media Thickness (CIMT) and Carotid Artery Stiffness

CIMT is a structural measure of arterial wall thickness (tunica intima and tunica media – the two innermost layers of the arterial wall). It is a measure of the potential presence of atherosclerotic disease in the arterial wall. Carotid artery stiffness is another measure of vascular function that is easily obtained at the time of CIMT assessment. Given variation in measurement technique and conditions, normal values do not currently exist.

Methods: B-mode ultrasound with a high resolution linear array vascular transducer will be used to record common, bulb and internal CIMT. A Meyer's arc[168] will record the angle at which the greatest CIMT is obtained so follow-up imaging can be obtained at the same site. Images will be stored electronically and sent to the core lab for reading. Automatic edge detection software will be used with 3 readings averaged for analysis. For carotid artery stiffness, an optimal 2-dimensional image of the common carotid artery will be obtained, and the M-mode cursor will be placed 1 cm proximal to beginning of the carotid bulb. The maximal and minimal lumen diameters will be read from the M-mode tracing for calculations of carotid artery stiffness. Calculations of carotid artery stiffness will include incremental elastic modulus (Einc),[229] Peterson mean pressure-strain elastic modulus (PEM),[230]Young's elastic modulus (YEM),[230] and beta arterial stiffness index (Beta).[230]

Training: CIMT and carotid artery stiffness assessment will be performed at each site by a trained ultrasonographer according to published pediatric guidelines.[228] Detailed training will be provided by webinar conducted by the vascular core lab. To insure standardization, each site will send up to 5 de-identified CIMT and carotid dimension images for review prior to study initiation, at regular intervals throughout the study, and whenever there are site personnel changes. Images acceptable for reading at all 3 sites (common, bulb, internal carotid) on both sides will be required for certification.

Quality Control: A core lab study (personal communication; Urbina E) involving more than 800 participants showed that the coefficient of variation ranged from 1.8 (peak systolic dimension) to 5.5% (internal carotid) for within visit measures, which is acceptable according to published standards.[168] Every 6 months, reproducibility for the 3 readings of the carotid ultrasound for both (QC) CIMT and stiffness will be calculated by the DCC. Re-training for each technician via video-conferencing or at in-person PHN steering committee meetings will be provided as needed. Sites will be instructed to send 1 practice study per technician every 6 months for review and webinars will be conducted prior to year 2 data collection.

4.3.2 Measures of Secondary Outcomes

Laboratory Assessment

Muscle Toxicity: CK will be measured by each center's clinical laboratory or at a certified local lab to assess potential statin effects on muscle inflammation. While CK levels do vary by clinical laboratory, as well as ethnic origin, age and gender (higher in younger African American men),[231] pediatric statin trials in FH and adult trials have defined abnormality using a single safety cutpoint of >10 times the upper limit of normal for a study site's clinical laboratory. Symptoms will also be recorded at each study visit, and participants will be instructed to report any symptoms to study personnel that occur between visits. If symptoms or CK elevation are noted, assessment for a clinical explanation (e.g. muscle trauma/injury/excessive exercise) will be made, and consideration will be given to temporarily withholding study drug followed by reassessment.

AST and ALT: AST and ALT will be measured by each center's clinical laboratory or at a local certified lab to assess potential statin effects on the liver and to monitor for development or progression of NAFLD. For participants without known liver disease (other than NAFLD) or ALT elevation at randomization, increases in ALT will be assessed and managed as per the algorithm outlined in the table in **Section 5.3.1**. Participants with persistent ALT abnormalities, abnormal bilirubin or INR, or symptoms will be further assessed by the site's gastroenterologist/hepatologist, and the results of any additional evaluation will be recorded and reported.

Diabetes and Glucose Homeostasis: Fasting glucose and HbA1c will be measured by each center's clinical laboratory or at a certified local lab to assess potential statin effects on incident diabetes and to monitor for the development or progression of impaired glucose homeostasis/type 2 diabetes. Additionally, insulin and C-peptide will be measured at baseline, 6, 12, 18 and 24 months after randomization. Surrogate markers of insulin sensitivity (1/Fasting insulin, HOMA-IR, QUICKI) will be calculated at these assessments.[107,108,232, 233] An HbA1c ≥6.5% or a fasting glucose ≥126 mg/dL suggestive of diabetes will be further evaluated as directed by each center's endocrinologist, and the results of any additional evaluation and therapy will be recorded and reported.

Lipid Measures

Standard Fasting Lipid Profile (FLP): A standard FLP measures total cholesterol, TG, and HDL-C, from which non-HDL-C can be calculated and LDL-C can be accurately calculated provided TG levels are <400 mg/dL. FLP remains the cornerstone for assessment of cardiovascular risk and guidance of primary prevention therapies.[234] FLP will be obtained at all study visits and measured in the core lab. Total cholesterol and LDL-C results will not be made available to clinicians as the results could lead to unmasking.
Methods: Total cholesterol, TG and HDL-C will be measured at a centralized core laboratory using enzymatic assay, with calculation of LDL-C via the Friedwald equation, and direct LDL-C measurement if the TG level exceeds 400 mg/dL. LDL-C (direct) is measured by a homogeneous enzymatic assay.

NMR Spectroscopy Lipoprotein Particle Assessment: Lipids are carried within lipoprotein particles that are heterogeneous in size, density, charge, core lipid composition, specific apolipoproteins and function. Knowledge of the full lipid phenotype will inform the understanding of the CVD risk associated with CDO. Beyond the alteration of lipoprotein metabolism visible on the standard lipid profile (TG and non-HDL-C elevation and HDL-C reduction), extensive interrelated changes in lipoprotein subclass levels and particle size distributions are predictive of cardiometabolic risk.[235] NMR spectroscopy assessment will be performed at baseline, and 6, 12 and 24 months after randomization.
Methods: The lipoprotein particle concentrations and size will be batched and measured on frozen plasma specimens (−70^{o}C) by proton NMR spectroscopy at a centralized core laboratory. Particle concentrations of lipoprotein subclasses of different sizes will be directly obtained from the measured amplitudes of their spectroscopically distinct lipid methyl group NMR signals.[236] VLDL, LDL, and HDL particle subclasses of varying size will be quantified from the amplitudes of their spectroscopically-distinct lipid methyl group NMR signals. VLDL-P, LDL-P, and HDL-P are the totals of the particle number concentrations of their respective subclasses and their weighted-average particle sizes will be calculated from the sum of the diameter of each subclass multiplied by its relative mass percentage estimated from the amplitude of its methyl NMR signal.[236]

Apolipoproteins AI, B, and CIII (apoAI, apoB, and apoCIII): The physiologic functions of lipoproteins are dependent on multifunctional apolipoproteins that serve as templates for their assembly, maintain their structure, direct their metabolism through binding to membrane receptors and regulate associated enzyme activity.[237,238] ApoB specifically relates to CVD risk attributed to apoB-containing lipoprotein particles, and the proatherogenic/antiatherogenic ratio of apoB/apoAI may be an even better risk discriminator than either apoB or LDL-P.[237, 239] Because postprandial lipid metabolism is increasingly recognized to be relevant to the atherogenesis of obesity and insulin resistance, measurement of intestinal apoCIII confers

crucial information to the evidence-base on the atherogenicity of downstream LDL-P in CDO. Hepatic apoCIII synthesis and secretion also plays an important role in determining the size or number of TG-rich VLDL particles secreted by the liver and so determines the concentration of circulating, atherogenic VLDL, IDL, and small LDL particles,[239,240] and appears to compromise HDL antiatherogenic functionality.[239] Apolipoproteins will be measured at baseline, and 6, 12 and 24 months after randomization.
Methods: Apolipoprotens will be measured at a centralized core laboratory on batched frozen plasma specimens by immunonephelometric assays with international standards applied.[241]

4.3.3 Covariate Measures

Family History

Rationale: Family history of premature CVD may be a marker of an underlying genetic dyslipidemia, or a more complex genetic and environmental background (including other CVD risk factors) that may influence progression of atherosclerosis.

Methods: Family history will be obtained from the parent/guardian at the screening visit and updated at all subsequent visits. A questionnaire will assess the presence of premature CVD, as defined as myocardial infarction, angina, stroke, or CVD procedures (CABG, coronary artery angioplasty and/or stent placement) occurring before the age of 55 years in male relatives and before the age of 65 years in female relatives, in first degree (parents, siblings) and second degree (aunts, uncles and grandparents).

Lifestyle Assessment

Rationale: Lifestyle changes aimed at ultimately reducing adiposity, improving insulin sensitivity and reducing lipid substrate are the primary treatment modalities for addressing CDO, although sufficient changes are rarely achieved. Changes in both diet (reduction in sugars, simple carbohydrates and fats) and exercise can improve both triglyceride levels and HDL-C levels both independently and in association with reductions in adiposity, although this may be insufficient to reduce LDL particle numbers or improve vascular measures. In addition, poor sleep quality is prevalent in adolescents, and obese participants in particular, on a spectrum that ranges to obstructive sleep apnea, and has been associated with an adverse impact on CV risk factors, particularly blood pressure and non-HDL-C.[242] Changes in sleep quality have not been well studied in this population.

Methods: Dietary assessment and lifestyle behaviors will be assessed by completion by the participant with study personnel of the Preventive Cardiology Lifestyle Screener questionnaire (DeFerranti, Boston, MA) and the Pittsburgh Sleep Quality Index[242,243] at randomization, 6, 12 and 24 months. Each participant will be provided with a physical activity tracker device, from which 7 days (5 weekdays, 2 weekend days) of data will be obtained following randomization prior to the visit at 1 month, and preceding visits at 6, 12, and 24 months. Data collected will include sleep, daily steps, and daily number of activity minutes, averaged over the 7 days of device wear.

Socioeconomic Status

Rationale: Several studies have highlighted an association with lower socioeconomic status and a greater prevalence of obesity and adverse lifestyle behaviors.[244,245]

Methods: Socioeconomic status will be assessed indirectly by linkage of census tract indicators with participant zipcode or postal code of residence. It will also be assessed by obtaining maternal highest level of education at the baseline visit.

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Anthropometry, Blood Pressure

Rationale: BMI, calculated from height and weight (using CDC growth charts), remains the standard, validated proxy for adiposity, rarely confounded by muscle mass. Ectopic adiposity is more relevant to cardiometabolic risk than absolute adiposity but BMI predicts insulin resistance in adolescents as well as waist circumference, the accepted surrogate measure of visceral adipose tissue.[246] The waist to height ratio, however, can more accurately predict cardiometabolic risk in adults[247] and adolescents.[248] Anthropometry will be assessed at screening, baseline, 3, 6, 12, 18 and 24 months. The presence of acanthosis nigricans is felt to be an important physical marker of metabolic risk, particularly insulin resistance and diabetes, in obese youth. It will be assessed at the baseline visit only. Sexual maturation will be assessed by Tanner staging to be performed at baseline, and at 6, 12 and 24 months after randomization. Blood pressure is a cardiometabolic risk factor also known to be associated with adiposity, and independently can influence vascular measures, and will be assessed at every visit.

Methods: Height (centimeters) will be obtained using a standardized stadiometer and weight (kilograms) will be obtained using a calibrated scale. While equipment will not be provided and is available at all sites, there should be no changes in equipment used at sites throughout the trial. Measurements will be made 2-3 times each and averaged. BMI (kg/m^2) will then be calculated and converted to age- and sex-matched percentiles based on data from the Centers for Disease Control (CDC; http://www.cdc.gov/nchs/data/series/sr_11/sru_246.pdf). Scales will be calibrated by the sites every 6 months. Waist circumference will be measured at the top of the posterior iliac crest according to NHANES standards, and normalized by calculating waist to height ratio.[248] A Dynamap oscillometric device may be used to measure systolic, diastolic, and mean arterial pressure and simultaneous heart rate in triplicate. Established guidelines for blood pressure measurement will be followed, with measurement in the right arm, using a cuff of the appropriate size with the arm at heart level after 5 minutes of resting with the participant seated.[147, 249] Oscillometric readings that are above the 90th percentile for gender, age and height will be repeated with an auscultatory technique. Tanner staging will be measured by validated self-assessment questionnaire utilizing realistic color photographs of gender-specific Tanner stages.[250-252]

High Sensitivity C-Reactive Protein (hs-CRP)

Rationale: CRP is increased in persons with insulin resistance, hypertension, CVD, and type 2 diabetes.[253] CRP is higher in overweight children compared to normal weight children,[254] and is associated with insulin resistance and cardiovascular risk factors including the metabolic syndrome.[255,256] CRP may promote arterial inflammation in the vessel wall, and may prevent formation of nitric oxide by endothelial cells, which could promote vasoconstriction and up-regulate angiotensin receptors.[257] The reduction in cardiovascular events seen with statins, which have anti-inflammatory effects, and the reduction in CRP with statin treatment, further support a causal role of inflammation.[258] Thus, CRP may be a potential effect modifier/covariate of statin treatment on vascular outcomes.

Methods: Blood samples for hs-CRP plasma concentration will be obtained at baseline, and at 6, 12 and 24 months after randomization.

DNA Banking

DNA samples will be banked at a centralized core laboratory at baseline and at 24 months after randomization, together with EDTA plasma, for future analyses including epigenetics (a CBC will be obtained at 24 months only) and responses to treatment.

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Rationale: Patients with elevated but not extreme dyslipidemia can have an increased number of a diverse group of genes associated with abnormal lipid levels and can have "balancing" genetic variants associated with adverse and protective lipid effects.[259] Separate groups of genes associated with LDL-C, triglyceride metabolism,[260] obesity and diabetes are all associated with the dyslipidemia to be treated in this study. Genetic characterization of this cohort will be helpful in understanding the common genetic variants associated with the dyslipidemia of adolescent obesity. Comparisons with genetic studies of older cohorts will add information on the long-term natural history of this dyslipidemia.

Genetic variants can also be informative in discerning both variable responsiveness to statins and susceptibility to adverse side effects. In particular, variants in the cytochrome P450 (CYP), UDP-glucuronosyltransferase enzymes and organic anion transporters (OAT1 and OAT3) gene families involved in statin metabolism and the ABC-transporter pathway for statin biliary elimination can all play a role in the impact of therapy.[261-263] The more hydrophilic compounds require active transport into the liver, are less metabolized by the CYP family, and exhibit more pronounced active renal excretion via organic ion transport. Pitavastatin, while it is a lipophilic statin, is less metabolized by the cytochrome p450 pathway.[264] Genetic alteration of OAT1 and OAT3 pathways that affect statin levels are also reflected in plasma concentrations of multiple organic ions that share the same clearance pathway.

Epigenetic modifications that influence the expression of genes, such as DNA methylation, are emerging as factors that contribute to variation in lipid traits. Recent evidence from the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study showed methylation of *CPT1A*, a key enzyme in beta-oxidation of long chain fatty acids, was highly associated with triglycerides.[265] It is reasonable to investigate whether risk stratification could be improved by assessing the contribution of baseline DNA methylation to the response to statin treatment, and additionally whether the alterations in DNA methylation result from standard of care lifestyle treatment or statin among adolescents with obesity-related dyslipidemias. Commercially available high throughput DNA methylation microarrays are available and can be performed on stored DNA samples.

Plasma/Serum/Urine Banking

Plasma/serum samples will be banked for future analyses at baseline, and at 6, 12, 18 and 24 months after randomization. Urine samples will be banked for future analysis at baseline, and at 6 and 24 months after randomization.

Rationale: Banked plasma/serum/urine samples may be used to explore possible covariates that may either be associated with CDO and/or with the vascular measures, or may influence or be influenced by statin treatment. For example, low vitamin D has been associated with increased myopathy with statins, is reported to be prevalent in obese youth, has been linked to insulin resistance and is associated with increased vascular stiffness.[266-269] Hyperuricemia may influence blood pressure which may influence PWV. Adolescents in the highest tertile of serum uric acid levels are more likely to have high blood pressure and other components of the metabolic syndrome.[270] Serum uric acid may be in the causal path for vascular stiffening through activation of the renin-angiotensin system, and decreased nitric oxide production.[271] The effect of statins on uric acid is unclear; some studies show increases in uric acid while others show decreases.[272,273] A distinct apolipoprotein(a) [Lp(a)] that resembles plasminogen binds to apolipoprotein B containing apolipoproteins, especially small LDL isoforms because of their longer half-life, conferring atherothrombotic risk. Measurement of Lp(a) is recommended for "individuals with an increased risk of CVD, particularly in those with borderline LDL-C or high apolipoprotein B" which characterizes the high LDL-P phenotype of CDO.[274] Therapeutic options

are limited but lifestyle can have a positive impact in adults (11.3% reduction) and warrants further study in pediatrics.[275] Adipose tissue is recognized as an important secretory organ, producing a range of bioactive proteins, collectively called adipokines, whose dysregulated production participates in the pathogenesis of obesity-associated co-morbidities. Increased levels of leptin and/or leptin resistance[276] and decreased adiponectin are related to insulin resistance, overweight and obesity. The finding that obese individual have increased appetite and decreased energy expenditure upon weight loss and that these changes are blunted by the administration of leptin suggests that a leptin setpoint may undermine maintenance of long term weight loss but improved insulin sensitivity may improve leptin sensitivity.[277] Hyperinsulinism in insulin resistance lowers adiponectin receptors blunting the potent endothelium-protective effects exerted by adiponectin. Low adiponectin concentrations are inversely associated with CRP and tumor necrosis factor α (TNF-α) levels.[278] Thrombosis is a complication of obesity;[279] elevated PAI-1 and fibrinogen reflect a state of increased thrombotic potential contributing to the development of vascular complications.[280] An excessive amount of lipid stored in adipocytes leads to functional abnormalities of the endoplasmic reticulum and mitochondria, which, in turn, contribute to intracellular and systemic disorders such as the stimulation of a proinflammatory state due in part to high production of free fatty acids that accumulate in nonadipose tissue and impair insulin signaling and glucose tolerance.[281] In addition to CRP, leptin and adiponectin, the major mediators responsible for the balance between proinflammatory and anti-inflammatory pathways include TNF-α, interleukin-6 (IL-6), serum retinol-binding protein 4 (sRBP-4) and plasminogen activator inhibitor 1 (PAI-1). There is a strong relationship between TNF-α and the effect of lipoprotein lipase, which is responsible for the breakdown of circulating triglycerides and VLDL. In metabolic syndrome, there is an increase in macrophage TNF-α expression and a decrease in the effect of lipoprotein lipase, thereby causing hypertriglyceridemia. TNF-α also plays a role in stimulating the expression of other inflammatory mediators, such as IL-6, and reduces the expression of anti-inflammatory mediators, such as adiponectin. sRBP-4 is a circulating protein of the lipocalin family associated with visceral adiposity and insulin resistance. sRBP-4 is secreted by the liver and adipocytes, reaching high concentrations in chronic low-state inflammation. In humans, a number of studies report an association between sRBP-4, insulin resistance, and type 2 diabetes.[282] PAI-1 is a member of the serine protease inhibitor family and is the primary inhibitor of fibrinolysis. It is secreted mainly by platelets and vascular endothelium and is also produced by adipocyte cells. Plasma PAI-1 is elevated in individuals with obesity and plays a key role in promoting thrombus formation following the rupture of atherosclerotic plaque.[283] Vascular oxidative stress is an established complication of obesity but is challenging to quantify due to the immediacy of redox metabolic reactions, but plasma F2-isoprostanes are a stable byproduct of the reaction of free radicals with arachidonic acid.[284] Considered the most reliable biomarker for lipid peroxidative stress in vivo, F2-isoprostanes are independently associated with coronary heart disease.[285] 90% of plasma F2-isoprostanes resides on lipoproteins, predominantly HDL.[286]

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4.3.4 Schedule of Measurements

Table 1. Schedule of Visits/Measurements (months from randomization)

Measurement	Timepoint/Month						
Timepoint/Month	SV^	0	1	6	12	18	24
Vascular measures (PWV, CIMT, carotid stiffness)		X		X	X		X
Fasting lipid profile^^	X	X	X	X	X	X	X
Lipid NMR spectroscopy, apolipoproteins		X		X	X		X
Metabolic panel, urinalysis, TSH, fasting glucose, HbA1c*, CBC**	X						
Urine pregnancy test¥	X	X					
AST, ALT	X	X	X	X	X	X	X
Glucose homeostasis - fasting glucose, HbA1c		X	X	X	X	X	X
Glucose homeostasis – insulin, C-peptide		X		X	X	X	X
CK, muscle symptoms	X	X	X	X	X	X	X
Clinical and family history, pregnancy/contraception counseling	X	X	X	X	X	X	X
Anthropometry, blood pressure	X	X		X	X	X	X
Lifestyle assessment		X		X	X		X
Socioeconomic status		X					
Tanner staging (self report)		X		X	X		X
Adverse event assessment		X	X	X	X	X	X
hs-CRP		X		X	X		X
DNA banking (+CBC at 24 months only)		X					X
Plasma/serum banking		X		X	X	X	X
Urine banking		X		X			X
Estimated amount of blood draw (mL)	7	37	11	34	34	31	37
Estimated visit duration (hours)	1-2	2-4	1-2	2-4	2-4	1-2	2-4

^ SV = screening visit (interval between SV and randomization minimum 2 weeks; maximum 6 weeks); Study visit windows for month 1 visit is ±2 weeks, month 6 to 18 are ±4 weeks, and month 24 is ±6 weeks.

^^ Fasting interval for screening visit and core lab measures is a minimum of 10 hours.

* HbA1c must be done at a site lab and not point of care.

** CBC differential must include absolute counts of both segmented and band neutrophils.

¥ After screening and baseline, urine pregnancy tests may be performed at subsequent times at the discretion of site study personnel.

4.4 Study Visits

There will be a total of 7 study visits. Potentially eligible participants identified from medical records and clinic rosters will be reviewed and a screening visit will be scheduled. Fully eligible and consenting participants will then be scheduled for the baseline/randomization visit at least 2 weeks and no more than 6 weeks from the screening visit. Additional contact with participants between study visits will be at the discretion of the site personnel and will include contact for scheduling; such potential contact will be described in the informed consent form. Female

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participants will be counseled about pregnancy and appropriate methods of contraception at every study visit. Since participants will be fasting for at least 10 hours at the time of each blood draw, refreshments will be provided.

5. SELECTION AND WITHDRAWAL OF PARTICIPANTS

5.1 Participant Inclusion Criteria

STEP 1 (Pre-screening visit): Potential participants will meet all of the following criteria based on medical record review or from a visit within the previous 6 months before being considered for screening (if known exclusion criteria, such as elevated TSH, are noted at this step and cannot be clarified the patient will be excluded from STEP 2):

- Boys and girls ages 10 to 18 years inclusive
- BMI ≥85th%ile (CDC BMI charts)
- Fasting lipid profile (FLP) within 6 months at the clinical site or outside lab with all of the following criteria met:
    - LDL-C <160 mg/dL and ≥90 mg/dL, and
    - TG <500 mg/dL, and
    - TG/HDL-C ratio ≥2.5 or HDL-C <45 mg/dL for boys or HDL-C <50 mg/dL for girls, and
    - non-HDL-C ≥125 mg/dL

STEP 2 (Screening visit): Potential participants who meet pre-screening criteria will be assessed at a screening visit after at least 2 weeks and not more than 6 months from the pre-screening assessment, and must meet the following inclusion criteria before being considered potentially eligible for participation:

- Boys and girls aged 10 to 18 years (with 2 year availability for study participation)
- BMI ≥85th percentile
- FLP performed at the clinical site with all of the following criteria met:
    - LDL-C <160 mg/dL and ≥90 mg/dL, and
    - TG <500 mg/dL, and
    - TG/HDL-C ratio ≥2.5 or HDL-C <45 mg/dL for boys or HDL-C <50 mg/dL for girls, and
    - non-HDL-C ≥125 mg/dL
- Participant consent, or parental/guardian consent and participant assent

5.2 Participant Exclusion Criteria

Potential participants who meet screening criteria will be assessed at a screening visit, and must not have any of the following exclusion criteria before being considered potentially eligible for participation:

- Current use of lipid lowering medication, antihypertensive medication, growth hormone, systemic corticosteroids, cyclosporine, protease inhibitors, erythromycin, rifampin, colchicine, warfarin, second generation psychotropic drugs, isotretinoin; stable doses of stimulant or antidepressant therapy will be accepted
- Known allergy or hypersensitivity to statin.
- Patients who have had bariatric surgery or plan to have bariatric surgery during the trial
- Female who is pregnant, plans to become pregnant or is sexually active without contraception
- Stage 2 hypertension (systolic or diastolic blood pressure ≥95th percentile for age, sex and height percentile + 12 mmHg, or ≥140/90, whichever is lower; confirmed after an

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appropriate evaluation) [Potential participants with screening BP values suggestive of stage 1, pre-HTN or white coat HTN will be evaluated to exclude that they do not indeed have stage 2 HTN]

- Diabetes (type 1 or type 2) by ADA criteria (fasting glucose ≥126 mg/dL, HbA1c ≥6.5%, random glucose ≥200 mg/dL, or 2-hr OGTT glucose ≥200 mg/dL)
- Use of insulin sensitizing therapy
- Known renal insufficiency (known chronic renal disease, estimated GFR <60 mL/min/1.73m^2 at screening, calculated using the Bedside IDMS-traceable Schwartz calculator for children = [0.41 x height in cm] / creatinine)
- Uncontrolled thyroid disease (TSH at screening >1.5x upper limit of normal, clinical of other laboratory evidence of hypothyroidism, or thyroid hormone therapy that has not been stable for 6 weeks prior to screening)
- Proteinuria suggestive of renal disease (more than trace together with an elevated urine protein:creatinine ratio as per local lab)
- Syndromic patients or patients with neurocognitive delay precluding adherence to study drug
- Liver disease other than NAFLD either diagnosed or suggested by ALT ≥40 U/L, or severe NAFLD indicated by ALT ≥200 U/L*
- Unexplained persistent elevated CK level >3 times the upper limit of normal
- Plans to leave the geographic area before completion of the anticipated 2 years of trial participation
- Any unstable medical or emotional condition or chronic disease that would preclude following the protocol or impact valid PWV measurement
- Admits to current smoking, current alcohol consumption

* At the screening visit, if the ALT <40 U/L then the patient is eligible. If the ALT is ≥40 and <80 U/L, then additional bloodwork is required. If the patient has a normal INR and bilirubin performed with 4 weeks of screening, and no signs or symptoms of liver disease, they are deemed eligible. Consideration should be given to obtaining a GI consult. If the ALT is ≥80 and <200 U/L then additional bloodwork performed within 4 weeks of screening is required, and a GI consult must be obtained. If the patient has a normal INR and bilirubin, no signs or symptoms of liver disease, and no liver disease other than NAFLD as determined by the GI consult, they are deemed eligible.

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5.3 Participant Withdrawal Criteria

5.3.1 Discontinuation of Study Drug

Indications for temporary discontinuation of study drug:

- For elevations in ALT and other liver abnormalities, please see the table below for specific guidance.

ALT <40 U/L at Baseline*	ALT 40-79 at Baseline	ALT 80 to 199 at Baseline with NAFLD
At baseline:		
No further work-up	Assess bilirubin, INR within 4 weeks; GI consult suggested within 3 months	Assessment by bilirubin, INR within 4 weeks; GI consult necessary unless done within previous 3 months
	No further work-up if bilirubin and INR normal, and no liver disease other than NAFLD	No further work-up if bilirubin and INR normal, no concerns on GI consult, and no liver disease other than NAFLD
During study:		
If ALT >80, obtain bilirubin, INR within 4 weeks, GI consult within 3 months unless previously done within 6 months	If ALT ≥ 2 times the baseline, obtain bilirubin, INR within 4 weeks, GI consult within 3 months unless previously done within 6 months	If ALT ≥2x the baseline, obtain bilirubin, INR within 4 weeks, GI consult within 3 months unless previously done within 6 months
Hold study drug if:		
Increase in ALT to ≥200 for > 2 weeks	Increase in ALT to ≥200 for > 2 weeks	Increase in ALT to ≥3 times the baseline for > 2 weeks
OR		
Any elevation in bilirubin >ULN	Any elevation in bilirubin >ULN	Any elevation in bilirubin >ULN
Any increase in INR >0.2 above normal	Any increase in INR >0.2 above normal	Any increase in INR >0.2 above normal
Symptoms consistent with liver disease (as assessed by GI or hepatologist) **	Symptoms consistent with liver disease (as assessed by GI or hepatologist)**	Symptoms consistent with liver disease (as assessed by GI or hepatologist)**
Assessment during hold:		
Reassess ALT, bilirubin, INR within 2 weeks, obtain GI consult if not previously done within 6 months	Reassess ALT, bilirubin, INR within 2 weeks, obtain GI consult if not previously done within 3 months	Reassess ALT, bilirubin, INR within 2 weeks, obtain GI consult if not previously done within 3 months
May restart if:		
No symptoms, no liver disease other than NAFLD, ALT <200, bilirubin and INR normal after 2 weeks	No symptoms, no liver disease other than NAFLD, ALT <200, bilirubin and INR normal after 2 weeks	No symptoms, no liver disease other than NAFLD, ALT <3x baseline, bilirubin and INR normal after 2 weeks
Permanent discontinuation at any time if:		
ALT remains ≥200, bilirubin >ULN, INR ≥0.2 above normal,	ALT remains ≥200, bilirubin >ULN, INR ≥0.2 above normal, GI	ALT remains ≥3 x baseline, bilirubin >ULN, INR ≥0.2 above

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GI consult confirms liver disease, including severe NAFLD	consult confirms liver disease, including severe NAFLD	normal, GI consult confirms liver disease, including severe NAFLD
Participant meets criteria for hold study drug at a subsequent occasion	Participant meets criteria for hold study drug at a subsequent occasion	Participant meets criteria for hold study drug at a subsequent occasion

* Baseline refers to the time of randomization.
** Symptoms will include appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia [>5%] in combination with elevations in liver enzymes.

- CK elevation >10 times the upper limits of normal or symptoms (muscle pain or weakness) without documented clinical explanation (e.g. muscle trauma/injury/excessive exercise). If CK elevation or symptoms resolve after up to 4 weeks of temporary discontinuation of study drug, then study drug may be restarted.
- The study drug may be restarted if the reason for cessation is muscle inflammation and improves within 4 weeks; however, if the reason for cessation recurs within 4 weeks of re-initiation, the study drug will be stopped permanently (see below).
- Guidance for discontinuation of study drug, and restarting study drug in the setting of liver abnormalities is given in **Section 5.3.1**.
- Incident diabetes as defined by ADA criteria (fasting glucose ≥126 mg/dL, HgbA1c ≥6.5%, random glucose ≥200 mg/dL, or 2-hr OGTT glucose ≥200 mg/dL). Meeting any of these criteria should trigger investigation by an endocrinologist. After investigation and at the discretion of the treating endocrinologist, the participant may either restart study drug or have permanent discontinuation of study drug (see below).
- Other adverse events that are thought to be related to the study drug, in the judgment of the study investigator in consultation with the DCC, PHN Medical Monitor and DSMB.
- If the participant is required to take a prohibited medication (protease inhibitor, warfarin, colchicine, erythromycin, rifampin, cyclosporine).

Indications for permanent discontinuation of study drug:

- Participant (or legal guardian) declines further medication participation but wishes to remain in the study.
- For elevations of ALT and other liver abnormalities, please see the table under **Section 5.3.1** for specific guidance regarding permanent discontinuation.
- Diagnosis of new liver disease other than NAFLD.
- CK elevation or symptoms without documented clinical explanation that return within 4 weeks of restarting study drug after temporary discontinuation.
- CK elevation or symptoms that persist for more than 4 weeks after temporary discontinuation of study drug.
- New onset diabetes, at the discretion of the treating endocrinologist.
- Participants who develop LDL-C ≥160 mg/dL during the trial continuously for at least 6 months will have permanent discontinuation of study drug, will be treated with a statin (preference will be pitavastatin 4 mg per day) at the discretion of the treating physician and will continue full participation in the trial.
- Participants who develop TG ≥500 mg/dL during the trial on at least 2 consecutive assessments will continue study drug and continue full participation in the trial, and may be treated at the discretion of their health care provider. If lipid-lowering medication is started, study drug will be permanently discontinued and the participant will continue full participation in the trial.
- Pregnancy or planning pregnancy.

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- Participant has bariatric surgery during the course of the study.
- Other adverse events that are thought to be related to the study drug, in the judgment of the study investigator in consultation with the DCC, PHN Medical Monitor and DSMB.
- If a participant requires prolonged use >4 weeks of a prohibited medication (protease inhibitor, warfarin, colchicine, erythromycin, rifampin, cyclosporine).

Study drug will be discontinued for each of these issues; however, there will be no unmasking until the end of the study unless requested by the DSMB or emergency situations. Consideration for withdrawal will trigger review by the Medical Monitor. The study drug may be restarted if the reason for cessation is liver or muscle inflammation and improves within 4 weeks; however, if the reason for cessation recurs within 4 weeks of re-initiation, the study drug will be stopped permanently. The reasons for temporary and permanent discontinuations of study drug will be documented. If the study drug is permanently discontinued, the participant will continue to be followed on the diet and lifestyle restrictions and undergo expected tests and measurements through the planned completion date (24 months after randomization), unless indication for withdrawal from trial is also met (see below).

5.3.2 Participant Withdrawal from the Trial

Participants may be withdrawn from the trial for the following reasons:

- Written participant/guardian refusal to continue in the trial, including refusal of the participant to sign the adult informed consent form upon reaching the age of consent.
- Participant non-compliance with study visits, assessments and study drug such that data from that participant are absent or of poor validity, despite every effort being made by site staff. The decision to withdraw a participant for non-compliance will be made jointly with the site investigators, DCC, and the study PI; input from the DSMB may also be sought. Reasons for withdrawal will be carefully recorded.
- Participant is diagnosed with an eating disorder .
- Participant develops a medical or psychiatric condition that precludes full participation in study interventions and measures.
- Participant undergoes bariatric surgery.

The reason and the circumstances of withdrawal will be documented for all participants withdrawn from the study.

5.4 Participant Availability

To establish feasibility for study recruitment, a survey and audit of clinical populations available at study sites (9 core PHN sites and 3 additional sites) was performed. Data for clinic volume, patient type and capacity were collected. Based on reported patient volumes, study site preventive cardiology and obesity clinics have 655 new and 1273 follow-up patient visits per month. To determine availability of potential participants eligible for this trial, the sites performed a chart/database audit for patients seen in preventive cardiology and obesity clinics within a 2-year period who met the following major eligibility criteria:

- Age 11-17 years
- BMI% ≥95th
- LDL <160 mg/dL
- TG/HDL ≥2.5
- non-HDL ≥145 mg/dL

Of the 12 sites surveyed, 3 PHN sites had sufficient detail in their records to apply additional exclusion criteria. From review of these 3 sites, it was noted that ~ 75% of those who met major

eligibility criteria actually met full study criteria for eligibility, consistent with a published statin trial in children and adolescents with FH.[184] Based on this information, it was estimated that 800 patients would be potentially eligible at screening, with 661 patients subsequently meeting full eligibility criteria. While study eligibility criteria have been modified since the audit, this was not expected to have a substantial impact on participant availability. To achieve the required sample size of 354 randomized participants, a consent rate of ~60% is necessary, which is potentially feasible and similar to that of other trials of drug therapy of pediatric dyslipidemia. If this consent rate is not achieved, then additional study sites may be recruited, some of which participated in the survey and audit. The sample size estimation has been inflated to include an hypothesized 20% drop-out proportion, which is conservative. The published statin trial had a 2% dropout during the placebo-controlled phase, with a total dropout rate of 7% at the end of the open label phase at 1 year. It is anticipated that there will be a higher proportion of dropouts in this trial compared to trials including patients with familial hypercholesterolemia, based on site PI's experiences with obese adolescents and the longer duration of this study.

5.5 Recruitment / Enrollment Procedures

Participants will be recruited from the participating PHN core sites as well as any auxiliary sites that join the study. The Principal Investigator or designee at each clinical center and the study coordinator will be responsible for case ascertainment and participant recruitment. Potential participants will be identified from patients who are referred to or who are already patients in Preventive Cardiology Programs and Obesity Programs at the study sites. Hence, prior to study launch, study staff will conduct informational sessions with all physicians, nurses, and other colleagues at their sites who care for the target population to describe the trial and solicit support for recruitment. Similar to previous PHN studies, information describing the study to help facilitate enrollment will be developed for print, electronic, and social media. Each site will negotiate ongoing involvement of clinical staff during participation in the trial regarding visits and measures, with a primary goal to prevent unintentional unmasking.

Study staff will review site databases/medical records for potentially eligible patients based on age, BMI and recent fasting lipid profile values (obtained within 6 months prior to review). In addition, all adolescent patients referred for clinical evaluation of dyslipidemia will be reviewed by study staff to determine whether they meet the criteria for screening. A screening log specifying reasons for exclusion will be kept and basic information will be reported to the DCC. Screening will continue throughout the study at participating sites to identify any additional participants that are new or were missed in the initial screening process.

Identified potentially eligible patients will be approached during a clinic visit, or, if eligibility is determined outside of a clinical visit, parents and potentially eligible patients will be contacted by study staff, as permitted by local IRB regulations, including mailing with opt-out card, a telephone call, email and/or other methods of approved initial contact. If a patient is interested in the study, the patient will be scheduled for the screening visit, and site personnel experienced in the treatment of dyslipidemia will be responsible for enrolling and consenting participants in person. If the patient declines study participation or does not attend the screening visit, this will be recorded in the screening log. At the screening visit and after consent has been obtained, each participant will be assigned a study identification (SID) number in order to keep study information confidential. The link between participant name and SID number will be stored only at the study site. Screening measures will be performed (clinical assessment including family history, smoking, medications and nutriceutical use, use of oral contraceptives, height, weight, blood pressure, complete metabolic panel, complete blood count, urinalysis, thyroid-stimulating hormone (TSH), pregnancy test, fasting lipid profile, fasting glucose and HbA1c) to confirm

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eligibility criteria. The consent form will state that all study procedures are conducted in accordance with local IRB or local national equivalent, NIH/DHHS, and HIPAA requirements of local and national laws and regulations. Participants who do not meet study eligibility criteria after screening assessment will be discontinued from study participation, and will not be scheduled for further study visits or measures. The screening data will be recorded and kept. Participants who meet eligibility criteria at the screening visit and provide informed consent will be scheduled for study visits.

6. TREATMENTS TO BE ADMINISTERED

6.1 Description of Study Treatments

Pitavastatin / Placebo

The study intervention will be randomization to pitavastatin versus placebo. Pitavastatin is the selected statin for this trial at a dose of 4 mg tablet per day with no titration; an identical placebo is available for the trial.

Rationale: Pitavastatin was chosen because: i) it is a potent statin with a significant impact on LDL levels per given dose; ii) two pediatric randomized clinical trials confirm lipid effects and safety in familial/ severe hypercholesterolemia (including recent PASCAL study);[148, 149, 184] iii) a study of adults, which included vascular stiffness showed benefit;[136] iv) has a good profile regarding adverse events; v) it is not metabolized predominantly through CYP450, eliminating potential drug and food interactions compared to other statins; vi) studies in adults with diabetes have shown a favorable risk:benefit profile.[212-214]

6.2 Medications/Treatments Permitted and Not Permitted during the Study

6.2.1 Rescue Medication, Emergency Procedures, and Additional Treatment(s)

Medical treatment for any non-cardiovascular or dysglycemic conditions is permitted during the trial. For dyslipidemia, hypertension, or diabetes see 6.2.2; for dietary and exercise treatments see special scenarios in 6.2.3.

6.2.2 Restrictions Regarding Concomitant Treatment

All medication, nutriceutical and concomitant treatments will be tracked at study entry and throughout the trial. Prohibited or discouraged concomitant treatments during the trial include:

- Lipid-lowering medications are prohibited at study entry and throughout the trial. Participants who develop LDL-C ≥160 mg/dL during the trial continuously for at least 6 months will have permanent discontinuation of study drug, will be treated preferably with a statin at the discretion of the treating physician (preference will be pitavastatin 4 mg per day if available, otherwise an available statin and dose of the treating provider's choice) and will continue full participation in the trial. Participants who develop TG ≥500 mg/dL during the trial on at least 2 consecutive assessments will continue study drug and continue full participation in the trial, and may be treated at the discretion of their health care provider. If lipid-lowering medication is started, study drug will be permanently discontinued and the participant will continue full participation in the trial.
- Anti-hypertensive medications are prohibited at study entry, but are permitted for treatment as per AAP guidelines should the participant develop hypertension during the trial.[249] The preferred medication for treatment will be lisinopril as a first line agent, and hydrochlorothiazide as a second line agent. Participants who begin taking anti-hypertensive medication will continue full participation in the trial, including study drug.

- Insulin and insulin-sensitizing agents are prohibited at study entry, but are permitted at the discretion of the treating physician for treatment as per American Diabetes Association (ADA) guidelines should the participant develop incident diabetes. Participants who begin taking insulin or insulin-sensitizing agents for diabetes or pre-diabetes/PCOS will continue full participation in the trial, including study drug.
- Use of vitamin supplementation (other than a daily multi-vitamin), products supplemented with plant stanols/sterols, and fish oil supplements is discouraged. Use of vitamin D supplements will be discouraged overall, but recorded at each study visit.
- Medications known to interact with statins are prohibited: cyclosporine, gemfibrozil, protease inhibitors, erythromycin, rifampin, warfarin, niacin, fenofibrate, and colchicine. Additional other prohibited lipid-lowering medications include statin, ezetimibe and red yeast rice extract.
- Use of oral isotretinoin is prohibited.
- Bariatric surgery is prohibited.

6.2.3 Restrictions on Diet and Lifestyle

All participants will receive their site's standard of care dietary and exercise intervention, and be monitored for lifestyle adherence as per the site's standard of care. The degree of family involvement regarding the standard of care lifestyle program will be at the discretion of the participant, family, clinical staff and study personnel. The site's lifestyle program will be concordant with the Expert Panel guidelines.[147] Compliance with the standard of care lifestyle intervention is at the discretion of the study participants. The following outlines specific contingencies that might arise during the trial:

- Dietary supplement use, particularly anti-oxidants, fish oil, and sterol/stanols, will be queried and recorded at each study visit, and may be reported by subjects between visits. Subjects are recommended not to take these supplements during their participation in the trial.
- Prohibited food items include grapefruit and grapefruit juice.
- If a reported diet as determined by standard of care assessment is identified as unsafe, diet counseling should be focused on remedying the unsafe components. Examples include inadequate micro-nutrient intake, a "fad" diet that is nutritionally incomplete, and severely hypocaloric diets.
- If dietary history identifies symptoms of bulimia nervosa or anorexia nervosa, appropriate referral will be made. If a diagnosis of anorexia nervosa or bulimia nervosa is made, then the subject will be withdrawn from the trial.
- If exercise history identifies unsafe exercise practices, subjects will be appropriately counseled.
- Development of an incident condition, either transient, such as an ankle sprain, or more serious that requires exercise restriction as part of treatment, will merit modification of lifestyle activity goals.
- Subjects who admit to current smoking and/or current alcohol use are excluded from study entry. Subjects who admit to current smoking and/or alcohol use during the trial will be counseled regarding smoking cessation and abstinence and will continue in the study.

6.3 Procedures for Monitoring Participant Adherence

Adherence with all study visits and measurements will be tracked, and reasons for non-adherence will be recorded. Study drug adherence will be assessed at each study visit by comparing the expected versus actual consumption of study drug tablets that were prescribed at

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the previous visit (study pharmacists will dispense the required amount to cover the interval to the next visit plus ensure that the participant has at least 2 weeks supply of overage,). The participant will bring all remaining study drug to the follow-up visit. The study coordinator or pharmacist will count and record the number of remaining tablets, and a new supply (depending on the visit schedule) will be dispensed according to site pharmacy practice. Unused study drug will then be returned to the pharmacy for disposal according to standard operating procedure. Study drug adherence will be measured as the percentage of study drug taken as prescribed over the interval between each visit, in the first 6 months and over the entire 24-month treatment period. Participants with <80% adherence over any interval will receive specific counseling from study personnel regarding compliance.

6.4 Procedures for Emergency Unmasking

An emergency code break will be available to the investigator / pharmacist / investigational drug storage manager. This code break may be used in emergency situations when the identity of the study drug must be known to the investigator in order to provide appropriate medical treatment or if code break is required to assure safety of study participants. Reasons to break the code might include clinical evidence of rhabdomyolysis, severe hypretriglyceridemia (confirmed TG levels >1000 mg/dL, or other important medical issues where care would be influenced by knowing whether or not the patient was taking a statin. If the code break for a participant is required, the DCC will be informed immediately. The reason for breaking the code will be documented in an appropriate eCRF along with the date and the initials of the person who broke the code. Non-emergent requests for unmasking must follow the procedure in the PHN Policy Manual and require advance request to the Trial Chair and/or the DCC.

6.5 Study Completion

Participants will be considered to have completed the study if they have completed all study measures for the final visit. The target window for the final visit is 24 months ± 6 weeks post-randomization. Further treatment of each study participant, including lifestyle and pitavastatin, will be directed by the participant's responsible physicians.

7. SAFETY ASSESSMENTS AND MONITORING

7.1 Specification of Safety Parameters

Given the study population and the potential for the presence of obesity-related co-morbidities which share similarities with adverse events (AE's) associated with statins, safety has been included as a secondary study aim, and is a prime consideration in this trial. The number and percentage of participants experiencing Adverse Events (AEs) and Serious Adverse Events (SAEs) will be summarized by system/organ class and by treatment group.

7.2 Recording and Reporting Adverse Events

A major component of safety monitoring is ascertainment and reporting of adverse events, including adverse drug reactions. The approach to these activities for this study is summarized in the sections that follow.

7.2.1 Definitions of Adverse Event, Suspected Adverse Reaction, Adverse Reaction and Unanticipated Problems The FDA Final Rule on IND Safety Reporting Requirements [http://edocket.access.gpo.gov/2010/pdf/2010-24296.pdf] provides the following definitions:

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- *Adverse event*: any untoward (e.g. unfavorable, negative, or harmful) medical occurrence associated with the use of a drug in humans, whether or not the event is considered drug related. An event can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the product.
- *Suspected Adverse Reaction*: any AE for which there is a reasonable possibility that the drug caused the event, meaning the event is possibly, probably, or definitely related to the study drug.
- *Adverse Reaction*: an AE for which there is a greater degree of certainty regarding causality; meaning the event is probably or definitely related to the study drug. Adverse reactions are a subset of Suspected Adverse Reactions.
- *Unanticipated Problem (UP)*: An UP is any incident, experience, or outcome involving risk to participants that meets all of the following criteria:
    - Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent formetc.)
    - Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
    - Suggests that the research places participants or others at greater risk of harm (including physical, psychological, economic, or social harm).

7.2.2 Classification of Adverse Events

Monitoring AEs requires that they be classified as to seriousness, expectedness, and potential relationship to the study drugs, all of which drive the reporting process.

a. Seriousness

Serious Adverse Event (SAE) is one that:

- Results in death,
- Is life-threatening (the participant was, in the view of the Principal Investigator, in immediate danger of death from the event as it occurred),
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
- Is a congenital anomaly/birth defect in the offspring of a participant, or
- Is an Important Medical Event that may jeopardize the participant or may require medical/surgical intervention to prevent one of the serious adverse event outcomes.

The Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 MedDRA 12.1 (http://ctep.cancer.gov) provides a grading system that is used to categorize the severity of adverse events, as follows:

Grade 1	Mild	transient, requires no special treatment or intervention, does not interfere with daily activities
Grade 2	Moderate	alleviated with simple treatments, may limit daily activities
Grade 3	Severe	requires therapeutic intervention and interrupts daily activities
Grade 4	Life-threatening Or disabling	
Grade 5	Death	

A SAE, as defined above, encompasses CTCAE grades 4 and 5, and any Grade 3 event that

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requires or prolongs hospitalization, or that substantially disrupts the ability of the participant to conduct normal life functions.

b. Expectedness
The purpose of reporting is to provide new, important information on serious reactions or events previously unobserved or undocumented. Therefore, all AEs will be evaluated as to whether their occurrence was unexpected, using the following definitions:

- *Unexpected*: An unexpected AE or adverse reaction is one for which the nature or severity is not consistent with information in the protocol, consent form, or product brochure. An AE or adverse reaction also may be categorized as unexpected if the event has not previously been observed at the same specificity and/or severity.
- *Expected*: An event is considered expected if it is known to be associated with the study drug(s) and/or the disease state. For this protocol, expected events reported since market introduction regardless of evidence of causality and included in the product brochure are:
    - Myalgia, muscle spasms, pain in extremity, back pain, arthralgia, rhabdomyolysis
    - Asthenia, hypoesthesia, fatigue, malaise, dizziness, insomnia, depression, headache, weakness
    - Diarrhea, constipation, dyspepsia, nausea, abdominal pain
    - Hepatitis, jaundice, fatal and non-fatal liver failure
    - Diabetes
    - Pruritis, rash, urticaria
    - Interstitial lung disease
    - Erectile dysfunction
    - Rare post-marketing reports of cognitive impairment

c. Causality
Causality assessment is required to determine which events require expedited reporting. The following criteria will be used to determine causality:

- *Not Related*: The event is clearly related to other factors, such as the participant's clinical state, or non-study drugs or interventions.
- *Possibly Related*: The event follows a compatible temporal sequence from the time of administration of the study drug, but could have been produced by other factors such as the participant's clinical state or non-study drugs or interventions.
- *Probably Related*: The event follows a reasonable temporal sequence from the time of drug administration, and cannot be reasonably explained by other factors such as the participant's clinical state, or non-study drugs or interventions.

7.2.3 Identification of and Data Collection Procedures for Adverse Events
AEs that are not considered adverse reactions or suspected adverse reactions will be identified when they are reported to the clinical center or during scheduled study visits by study coordinators and investigators. AEs will be assessed using self-report, physical examination data, and medical record review.

7.2.4 Reporting Procedures (Table 2)
Fatal or life-threatening AEs are to be reported to the DCC within 24-hours of first knowledge of the event. Those that are unexpected and considered possibly, probably, or definitely related to the study drug will be reported by the DCC to the FDA, the DSMB Chair, the MM, the NHLBI, and all study Investigators as soon as possible, but no later than 7 calendar days after first knowledge of the event, followed by a complete report within 15 calendar days. All other fatal or

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life threatening events that are unrelated to study drug will be reported semiannually to the DSMB and the NHLBI, and annually to the FDA.

All other *SAEs (i.e., non-fatal or not life-threatening)* that are unexpected and considered possibly, probably, or definitely related to the study drug will be reported to the DCC within 24-hours of learning of the event. The DCC will report the event to the FDA, NHLBI, DSMB and all study Investigators within 15 calendar days after first knowledge of the event.

All other *AEs* not meeting the criteria for expedited reporting will be reported to the DCC within 7 calendar days of first knowledge of the event. The DCC will report these AEs quarterly to NHLBI and annually to the FDA.

Table 2. Reporting of Adverse Events

Seriousness	Reporting Timeframe
Fatal or life threatening	Within 24-hours of learning of the event
Serious, but not fatal or life threatening, and pregnancy	Within 24-hours of learning of the event
All other	Within 7 calendar days of learning of the event

7.2.5 Reporting Adverse Events to Institutional Review Boards for NIH-Supported Multi-Center Clinical Trials

The site investigator or designee is responsible for reporting all serious adverse events to the local IRB in accordance with local policies and procedures.

7.2.6 Follow-up of Participants after Adverse Events

For AEs with a causal relationship to the study drug, follow-up by the Investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the Investigator.

7.3 Safety Monitoring

The Data and Safety Monitoring Plan for this trial will follow standard PHN monitoring principles. Oversight of data and safety is provided by the PHN Data and Safety Monitoring Board (DSMB), appointed by NHLBI. The DSMB meets at least twice a year to review data on AEs, adverse reactions, suspected adverse reactions, patient-reported outcomes, data quality, and study recruitment at regular intervals, and makes recommendations about study conduct to the NHLBI.

A DSMB Summary Report, summarizing the DSMB's findings, will be prepared within 30 days of each meeting and distributed to each Principal Investigator and Study Coordinator to be forwarded to the local IRB.

The DSMB and NHLBI are assisted by an independent Medical Monitor in reviewing serious adverse events in PHN studies. The PHN MM is the NHLBI's designee for determining causality and expectedness of all SAEs.

8. STATISTICS

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8.1 Statistical Analysis Plan

The primary analytic approach for all trial outcomes will be intention-to-treat (ITT). Several secondary analytic approaches will also be examined as outlined below and discussed in detail in a separate Statistical Analysis Plan (SAP).

- Non-intention-to-treat (NITT): Participants will be classified according to treatment actually received. NITT analyses, while potentially more powerful due to less dilution of treatment effect compared to an ITT analysis, are subject to bias because the inherent balance and lack of bias provided by randomization in measured and unmeasured patient characteristics of the two treatment groups is not preserved.
- Non-intention-to-treat by duration of pitavastatin use: If a substantial fraction of participants either cross-over from placebo to pitavastatin or are prescribed open-label pitavastatin or other statin therapy by their treating physicians, analysis will be conducted with 'time-under-statin' as a continuous or stratifying (categorical) predictor of change in the outcomes.
- Exclusion of ineligible participants: Secondary analyses will be conducted after exclusion of participants who were later found to have been ineligible at the time of randomization.
- Covariate-adjusted analysis: adjustment for baseline (pre-randomization) factors known to be associated with a particular outcome can increase the efficiency of the treatment effect comparison. Such factors will be identified from the literature and used as covariates in the trial outcome analyses.

Descriptive statistics will be used to determine the distribution of values for all variables, and variables requiring transformation to meet analytic assumptions will be appropriately transformed. Data analysis plans will be keyed to the specific aims as follows, and incorporate appropriate methodology as described below.

8.1.1 Analysis of the Primary Outcome

The analytic approach for the Primary Outcome is summarized here. The detailed plan for all planned outcomes is provided in the Statistical Analysis Plan (SAP).

Primary Aim: To compare the effect of pitavastatin versus placebo on vascular measures in at least 354 adolescents with excess adiposity (body mass index [BMI] ≥85th percentile) and CDO (defined as high non-HDL-C + high TG/HDL-C ratio).

Primary Hypothesis: Over 2 years of observation, participants in the pitavastatin group will have a significantly different change over time (slope) in pulse wave velocity (PWV) than participants in the placebo group.

Study Measures: Pulse wave velocity (PWV); secondary measures include carotid intima media thickness (CIMT) and carotid artery stiffness.

Primary Analysis: Change in each of the vascular variables will be compared between groups across all study time points using SAS for Windows® (SAS Institute, Cary, NC).

8.1.2 Pre-Specified Sub-Group Analyses

It is of interest to determine whether the effect of statin therapy vs. placebo differs across subgroups. The pre-specified subgroups for analysis of all primary and secondary outcomes are:

- Age at baseline (stratified by deciles)
- Gender
- Race
- Baseline insulin resistance (HOMA-IR) (stratified by deciles)
- Baseline BMI percentile (stratified by deciles)
- Family history of premature CVD

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Subgroup by treatment group by time interaction tests will be performed to test whether the treatment effect is homogenous across subgroups. Statistical testing within subgroup will not be conducted unless the interaction test p-value is ≤ 0.05.

8.2 Number of Participants to be Enrolled

The sample size for the study will be at least 177 participants randomized per group (at least 354 total randomized). The hypothesis is that vascular status as assessed by PWV will be improved by statin therapy. Specifically, there will be a significant group (drug vs. placebo) by time interaction effect on mean PWV in a repeated measures analysis appropriately controlled for participant and clinical site specific random effects.

Although reports of longitudinal measures or clinical trials using PWV in youth are nonexistent, our investigators[50] reported data from lean and obese youth age 10-24 years who were age, race, and sex matched using the same SphygmoCor device proposed for this study. In 241 lean participants (BMI <85th%ile), carotid-femoral PWV was 5.4 (0.7) compared with 6.3(1.1) in 234 non-diabetic obese participants (BMI ≥95th%ile). Obese youth had PWV 14% greater than lean youth. Two statin trials used PWV measured by the same SphygmoCor system to provide baseline and end of study vascular data for adults (average age 50s). In 20 adults with rheumatoid arthritis treated with simvastatin for 6 weeks, PWV dropped from 9.6 (2.3) at baseline to 8.9 (2.1), a 7.4% decrease from baseline.[287] In 35 hypercholesterolemic adults treated with rosuvastatin plus low-fat diet for 4 weeks, PWV dropped from 9.5 (1.9) to 7.8 (1.5), a decrease of 18% from baseline.[164]

Sample size was computed under several scenarios based on different values for the group by time interaction effect size in the 2 treatment groups (statin and placebo/no drug) assuming baseline PWV 6.3±1.1 m/sec and, based on unpublished data from Urbina et al. collected 5-years apart on 268 obese youth, a correlation within participants for repeated measures over time of 0.5 [observed value from Urbina et al. was 0.68]; alpha 2-sided = 0.05, power (1-beta) = 0.85, equal n for each group, equal assignment to treatment group at each clinical center and no stratification factors beyond treatment group, as follows:

Assumptions:

1. Baseline pulse wave velocity (PWV) is normally distributed with a mean of 6.3 m/sec and a standard deviation of 1.1 m/sec.
2. In addition to the baseline PWV measurement, PWV will be measured at after six months, 12 months, and 24 months.
3. Any change in PWV over the two-year duration of the study is conservatively assumed to be linear in time (i.e., 25% of the change will be observed at the six month visit, 50% of the change will be observed at the 12 month visit, and 100% of the change will be observed at the 24 month visit).
4. The following 3 hypotheses (scenarios) are considered:
    a. In Scenario 1, the PWV will remain unchanged in the control group over the two-year duration of the study and will decrease 8% in the statin group over the same 2-year period.
    b. In Scenario 2, the PWV will decrease 5% in the control group over the two-year duration of the study and will decrease 11% in the statin group over the same 2-year period.

c. In Scenario 3, the PWV will remain unchanged in the control group over the two-year duration of the study and will decrease 5% in the statin group over the same 2-year period.

5. The within-participant correlation between any pair of PWV measurements is assumed to be 0.5.
6. The sample size is adjusted by 20% to account for participant dropout.
7. The effect of crossover has not been incorporated into these sample size calculations.

Sample Size Calculations:

The mean slope (change) over two years of follow-up can be calculated as:

$$\mu_s = \overline{PWV}_{24} - \overline{PWV}_0$$

Where $\overline{PWV}_0$and $\overline{PWV}_{24}$ are the mean PWV at baseline and 24 months, respectively.

The variance of the slope over two years of follow-up can be calculated as:

$$\sigma_s^2 = \frac{\sigma^2}{SS_t}(1-\rho)$$

Where σ is the standard deviation of PWV (assumed to be constant over time for the purposes of these calculations), ρ is the within-participant correlation between pairs of PWV measurements, $SS_t = \sum(t_i - \bar{t})^2$, and $t = \{0, 0.25, 0.5, 1\}$ (since the assumed changes were given over two years of observation, it is easier to use this formulation for time).

After calculating the mean slopes for both the control and statin groups and the standard deviation of the slope, the Inequalities Tests for Two Means (Two-Sample t-Test) module of PASS was used to calculate the required sample size assuming a two-sided type 1 error rate of 5% and 85% power. The sample sizes required under each of the three scenarios are listed as follows:

Scenario	Δ_c	Δ_t	Slope_c	Slope_t	σ_s	N/group
1	0%	8%	0	-0.504	1.052	100
2	5%	11%	-0.315	-0.693	1.052	177
3	0%	5%	0	-0.315	1.052	253

Therefore, the conservative estimate of 11% decrease in PWV with statin treatment and 5% decrease in PWV with placebo at 2 years including 20% drop-out results in the need to randomize at least 177 participants per group for a total of at least 354 to detect an absolute difference of 6% in change in PWV between statin and placebo arms at an alpha of 0.05 and power of 0.85 for the group by time interaction. Assuming a crossover from placebo to open-label statin of no more than 5% by the end of the trial (from feasibility data from 9 sites, 22 of 454 potentially eligible participants had at least one LDL-C >160 mg/dL during follow-up and not continuously), this will result in a marginal dilution of the treatment effect such that these sample size estimates ultimately apply to an absolute difference of approximately 5.94% in change in PWV between treatment and placebo. It should be noted that the sample size of 177 randomized participants per group is conservative since recent data from Cincinnati suggest that non-diabetic obese participants on no drug therapy will have progression of PWV around 0.05 m/sec/year or 0.1 m/sec over the 2-year grant (presented at American Society of Hypertension meeting May 2013). Therefore, it is likely that the placebo group will show no improvement in PWV or more likely, progression (worsening; increase) in PWV.

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A secondary hypothesis is that the carotid bulb (site of earliest disease) CIMT of the statin group will be lower than in the placebo group at study end. In adults, progression on placebo is about 0.01 to 0.02 mm/year[288] compared to improvement of 0.01 to 0.02 mm/year with rosuvastatin[289], which results in a mean difference of 0.04 mm over 2 years. Adult studies using a lifestyle intervention[290] and small studies of children have showed no effect of lifestyle on CIMT.[291, 292] The only pediatric study (mean age 13 years)[138] using a statin was conducted in children with FH. Over the 2 year study, the investigators observed progression of IMT of +0.005 (SD 0.044) in control and regression -0.01 (SD 0.048) in pravastatin treated youth. These are underestimates since the investigators averaged all 3 carotid segment measures, and the common carotid is known to demonstrate a slower progression than the bulb and internal carotid measures. Since a longitudinal study of 84 non-diabetic obese youth in Cincinnati had internal CIMT progression of 0.015 mm for 2 years (with SD 0.1) and the current study will use a potent statin, pitavastatin, a better estimate of effect size would be progression of 0.01 mm in the placebo group and regression of 0.02 mm over 2 years in the statin group, which yields a difference of 0.03 mm. Also, newer auto edge detection software as will be used in this study (as compared to manual trace used above) has smaller SD of 0.06 to 0.07.[293] Using the higher SD of 0.07, and assuming a correlation of repeated measures over time = 0.3, at a two-sided alpha of 0.05, to achieve power of 0.85 to detect a significant group X time interaction in CIMT would require 177 randomized participants per group (including 20% drop-out).

8.3 Level of Significance

The type I error probability for the trial treatment comparisons will be 0.05 (two-sided). No adjustment will be made to account for comparing the treatment groups with respect to more than one outcome variable. However, the report will note the number of comparisons made, and the possibility that when many outcomes are analyzed, it is not unexpected that one or more might have a statistically significant treatment difference by chance.

8.4 Interim Analyses and Stopping Rules for Termination of the Study

Interim Evaluation of Sample Size Assumptions
Unforeseen inaccuracies in design assumptions, in particular having to do with the estimated variance (SD), could lead to decreases in the trial's statistical power to detect treatment effects. Accordingly, a blinded analysis will be conducted, during enrollment to estimate the SD of the estimated slope in both treatment arms. This analysis will be conducted within the intention-to-treat framework under which the primary analysis of the primary endpoint will be conducted (**Section 8.1**).

To allow time for corrective action if necessary, after approximately 30% of participants have data collected from their 6-month visit, an interim analysis will be performed (estimated to occur between 12 and 15 months after study initiation depending on enrollment ramp-up). The DSMB will review and approve/modify proposed corrective actions (see SAP for details on proposed actions).

Interim analysis
In addition to routine data reviews of safety data an early evaluation of the treatment effect at the interim analysis will be implemented at the time when 50% of the total number of subjects will have 2 or more longitudinal observations per subject. A non-binding recommendation of early stopping for efficacy will be suggested to the DSMB if the estimate of the treatment effect obtained at the interim analysis is positive and the corresponding one-sided p-value is lower than a small reference value (corresponding to the value of the O'Brien-Fleming spending

function at the information fraction of 0.5). A non-binding recommendation of early stopping for futility will be suggested based on the conditional power analysis.

In the absence of the suggestions for early stopping or in the case when the DSMB decides to overrule the suggested stopping, the study is continued and treatment efficacy is evaluated at the second look (after completion of the study). Further details could be found in SAP.

Safety

It is possible that the DSMB may recommend stopping the trial early due to safety concerns. To monitor safety, adverse event rates will be compared between the two trial arms and reported to the DSMB. Early stopping rules are only guidelines; the DSMB may take a more global view of the trial during data monitoring.[294-296] To provide this broader perspective, the DSMB reports will include summaries of accrual, patient characteristics, adverse events, compliance rates with therapy, frequency of protocol violations, data quality, primary and secondary endpoints, other information as requested by the DSMB, and any unanticipated special problems that arise during the conduct of the trial.

8.5 Queried/Missing Data Procedures

Consistency checks and range checks will be built into the data management system. This will allow many errors to be identified and corrected at the time of data entry. Additional queries regarding any problems with data will be sent to site coordinators regularly throughout the course of the study. Sites will also be monitored during the study, especially for late entry/editing of data, missing data and protocol violations/deviations. Any data which are judged by the medical monitors to be or unattainable or unverifiable, and which cannot be resolved, will be set to missing.

The study report will indicate the number of participants who have missing data on each study endpoint. For covariate-adjusted analyses, the number of participants who have missing data on the covariates will be reported. All analyses will be participanted to sensitivity checks to determine what, if any, consequences arise as a result of missing data in the trial. If the missing data are deemed consequential (i.e. they either result in clinically meaningful changes in estimates of effects or in statistically significant changes in estimates of effects or if they appear to result from informative sources and/or induce bias in the analysis) multiple approaches to addressing the missing data will be applied with further sensitivity analyses and analysis of assumptions to select an appropriate method which may include simple approaches (e.g. imputation of last-observation- or last-rank-carried-forward) to model-based approaches (e.g. pattern-mixture models) to multiple imputation, with compensatory adjustments made to the analytic methods as appropriate.

Throughout the study, the rate, timing, and reasons for participant withdrawal will be monitored by site and treatment arm. Any site with a pattern of differential withdrawal by treatment arm will be queried. If necessary, retraining will take place or the site may be barred from enrolling additional participants to the study.

8.6 Participants to be Included in Analyses

All participants who remain eligible for and enrolled in the trial will be included in the analysis and their data will be analyzed by intention-to-treat in the group to which they were randomized unless otherwise specified.

9. DATA MANAGEMENT

An Electronic Data Capture (EDC) system will be used for the study that is designed to support reliable and secure data entry for clinical research purposes. The system also provides seamless integration of eCRFs and paper-based CRFs within a single protocol if desired; implementation of protocol amendments; and SAS and XML study data exports. All trial records referred to in the applicable Regulations will be retained for a period of 25 years. (See Part C, Division 5 of the Food and Drug Regulations [C.05.012] and as explained in **Section 2.8.6 d**) of the Guidance Document for Clinical Trial Sponsors: Clinical Trial Applications: https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/applicatin-submissions/guidance-documents/clinical-trials/clinical-trial-sponsors-applications.html).

9.1 Data Entry

Data can be entered directly from multiple study sites via a fully validated and 21 CFR Part 11 compliant, secure Web application and stored centrally. A configurable sample-based double data entry system is available. Data are entered by participant study identification (SID) number; names will not be linked with participant data in the database. Study sites will maintain records in secure areas linking the participant name with the SID assigned for the study. Study sites will have full access to their own data and be able to view these data remotely. Study staff will not be able to view participant data associated with other sites.

9.2 Data Validation and Monitoring

Integrated into the data entry system are real time validations, including both inter- and intra-instrument data checks. Inconsistent or questionable values are flagged during entry, and an edit report is automatically generated to the data entry client. These edit reports provide the information necessary to investigate any data entry errors or resolved questions regarding out-of-range or questionable values. Second level query tracking allows monitors and data managers real time access to unresolved queries as well as the date and time of query generation and resolution.

9.3 Data Security and Integrity

All data changes are written to an audit trail. The audit trail identifies the data item by table, column and key field. The entry includes the user, date and time, as well as the old value and new value. Both patient related data as well as trial configuration data are written to the audit trail. Data are saved at regular intervals during data entry to prevent loss of information in the event of a disruption of the Internet connection. In the unlikely event of a major disruption, a backup connection allows full access to the Data Management System (DMS).

Several levels of security are employed to ensure privacy and integrity of the study data, including the following: Study access requires use of assigned user names and passwords. Individual roles and access levels are assigned by the study data manager. Passwords are changed regularly. Web-based entry uses secure socket layer data encryption. Data will not be stored on laptop computers.

9.4 Biospecimen Tracking

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Specimen tracking is started from the time of receipt at the site, through shipment to the central biorepository, during handling at the biorepository, and through shipment to various core laboratories. Each specimen will be labeled with a bar-coded label identified by a unique specimen number that is different from the participant's unique study ID number. The master list linking the barcode numbers to the participant study ID numbers will be maintained under password protection in the data management system at the DCC. This blinding code system will maintain the confidentiality of the specimens yet allow linkage of the specimens with clinical study data for analyses.

10. QUALITY CONTROL AND QUALITY ASSURANCE

The DCC has primary responsibility for QC/QA activities of the phenotypic data. The DCC also requires that the sites complete certain QC activities, most of which are monitored by the DCC. The key QC/QA activities are:

- Development of a Study Manual;
- Clearly formatted and carefully constructed Case Report Forms(CRFs) with clear, up-to-date manuals of instruction;
- Sign-Off Procedures for all CRFs;
- Central protocol training and certification of all site data collection staff with the use of standardized checklists;
- Data management training and certification of site personnel completing data entry and/or data management;
- Verification of patient eligibility;
- On-going monitoring of all protocols/data collection activities;
- Completion of reliability and/or pilot studies for key measurements as appropriate;
- Inclusion of repeat measurements, as feasible, in the course of the study; and
- Monitoring visits to sites as required with pre-specified goals and/or remote monitoring activities.

The DCC may conduct site visits to the Core Laboratories and/or Biorepository to review QA and QC procedures and data transfer to the DCC. Review of central laboratory-related reports will be conducted at least monthly to identify overall, core-lab or site-specific problems in data or specimen acquisition and reporting of results.

11. ETHICS AND HUMAN PARTICIPANTS CONSIDERATIONS

11.1 Potential Risks and Protection from Such Risks

Adverse effects from statins are rare at standard doses but include myopathy and hepatic enzyme elevation. In the meta-analysis of statin use in children, evidence of hepatic enzyme elevation and muscle toxicity did not differ between the statin and placebo groups. The statins do not influence levels of the essential fatty acids necessary for early central nervous system maturation and have not been shown to affect neurodevelopment in adolescents.

11.1.1 Hepatic enzyme elevation: Elevation of hepatic enzymes is rare with statin use in children and has been reversible when it is reported. In this trial, a baseline hepatic panel will be measured before initiating treatment. Routine monitoring of hepatic enzymes and clinical assessment for muscle toxicity are outlined in the protocol as are rules for stopping the medication. The risk of adverse events increases with use of higher doses and this will be avoided in this trial by the use of a single, low starting dose with no up-titration.

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11.1.2 Drug interactions: Adverse events also increase with concomitant use of interacting drugs, primarily with drugs that are metabolized by the cytochrome P-450 system, the primary mode of statin metabolism, although this may be less of a concern with pitavastatin. Drugs that potentially interact with statin metabolism include gemfibrozil, fibrates, protease inhibitors, erythromycin, rifampin, cyclosporine, warfarin and niacin and colchicine. Participants who are chronically maintained on these medications will not be included in the trial. Participants will be advised about potential future medication interactions. Potential interaction with pitavastatin will be checked whenever any new medication is initiated.

11.1.3 Myositis: Creatine kinase (CK) will be measured at baseline as a representative level for each participant. CK levels vary widely with activity and this is especially true for children and adolescents. Participants will be instructed to report all potential adverse effects, especially muscle cramps, weakness, asthenia, and more diffuse symptoms suggestive of myopathy. Whenever potential myopathy symptoms are reported, the site PI will assess CK and the relation of symptoms to baseline assessment and recent physical activity prior to making a determination of whether or not study medication should be temporarily stopped. If CK is 10 times above the upper limit of reported normal, considering the impact of physical activity, the patient will be monitored for resolution of myopathy symptoms and any associated increase in CK off drug. Consideration of restarting statin is described in the protocol.
Rhabdomyolysis has very rarely been reported in adults on statin therapy, reported at 3 per 100,000 person-years. Rhabdomyolysis has not occurred in any of the pediatric trials, including trials with pitavastatin, but the total number of participants is too small to evaluate that risk.

11.1.4 Pregnancy: Statins have been identified as potential teratogens on the basis of theoretical considerations and small case series but the available evidence is far from conclusive. In fact, epidemiological data collected to date suggest that statins are not major teratogens. The actual risk for an exposed pregnancy seems to be small. Nevertheless, given the scarcity of available data, it is still advisable to avoid use of these drugs in patients who are planning pregnancy in order to reduce the risks as much as possible. In this trial, the need for abstinence or use of appropriate contraceptive measures will be discussed before any female participant is enrolled in the trial. Pregnancy or a planned pregnancy during the trial are exclusion criteria. In addition, this risk will be listed in the consent and the assent. Females of childbearing potential (post-menarchal) will have a point-of-care urine pregnancy test performed at the screening and the baseline visits (randomization) and otherwise as indicated by clinical history to confirm negative status. Positive pregnancy test results will be reported to either the participant or the participant's parents according to the local institution's policy. Written documentation of birth control measures will be required for female participants who are sexually active. Use of oral contraceptives or abstinence will be verified at each encounter. Oral contraceptives are not contra-indicated if medically appropriate. Counseling regarding contraception and pregnancy will be provided at every study visit, and pregnancy testing may be performed at the discretion of the site study physician.

11.1.5 Growth and development: Growth and development, especially normal sexual maturation, are often raised as concerns regarding use of statins in childhood and adolescence. Clinical trials have included both male and female children studied over the time of puberty and have shown no impact on sexual maturation or height velocity. In the meta-analysis of statin use in children, growth, development and sexual maturation were all normal. Nonetheless, height, weight, and BMI relative to normal growth charts, sexual maturation, and development will be monitored throughout the trial.

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11.1.6 Diabetes: In adult studies, statin therapy slightly increases the risk of new-onset diabetes. This risk is related to the intensity of therapy, the statin used and degree of LDL lowering. The mechanism(s) are postulated to be through impaired beta cell function and/or impaired peripheral insulin sensitivity. There are no data regarding statins and incident diabetes in pediatrics. Diagnostic biomarkers for diabetes and insulin sensitivity, including fasting glucose and insulin, HbA1c and C-peptide, will be measured serially throughout the study.

11.1.7 Study procedures: Risks associated with drawing blood from a vein include momentary discomfort and/or bruising. Infection, excess bleeding, clotting, and fainting are also possible, although very unlikely. There are no significant procedural risks associated with physical examination or vascular testing. There is some inconvenience and burden of completing the study visits and questionnaires and some participants may feel uncomfortable answering questions. There is a chance that participation in this study could cause psychological distress.

11.2 Confidentiality, Protection against Risks

Investigators will take all reasonable measures to protect the confidentiality of participants and their families, including the following:

Use of Participant Study ID numbers
Each participant is assigned a study identification number (SID). All interview and clinical research data are stripped of identifiers and labeled with the study number. The enrollment log with participant identifiers will be maintained at each site in a secured, locked location available only to the study staff. Samples for DNA will be stripped of the study SID at the laboratory and assigned specimen numbers that are linked to the SID. The informed consent form states that study data will be made available to the Data Coordinating Center (DCC) and NIH/NHLBI to ensure study safety and quality control. The participant's name and any other identifying information will not appear in any presentation or publication resulting from this study.

Reporting of Test Findings
If an incidental finding is found on a trial clinical test that would be expected to substantially affect clinical care, the PI or other qualified member of the research team will take full responsibility for disclosing the findings to the patients/parents, communicating with their primary health care provider with permission, or making appropriate referrals as indicated. The participant may choose to seek a second opinion and/or appropriate clinical care. This might change the participant's insurability and employability as it relates to the clinical finding only. The presumption is that detection of a potentially clinically significant finding will prove to be beneficial.

All analyses using results from measurements on stored de-identified samples of plasma/serum/urine/DNA will be based on anonymized datasets after linkage with trial data. No results from these measurements can be traced to the study participant, and hence cannot be disclosed to the study participant. The results of future tests on biological specimens will not be released to the participant/family. At the end of the study, the results of the genetic testing may be published for all the participants as a group, but it will not be possible to provide results for an individual participant and medical management will not be changed based on individual results. There is a reasonable possibility that no findings will result from this research effort. If findings are detected, it may be years before any utility of these findings is realized. Further, if samples are "anonymized" prior to release to other investigators for research, it may not be possible to trace the results back to the participant.

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Non-paternity information will be kept in the strictest confidence and will not be divulged to research participants or their families.

Certificate of Confidentiality: To help us protect the privacy of participants involved in this study, we will obtain a Certificate of Confidentiality from the National Institutes of Health (NIH). With this Certificate, the researchers of this study cannot be forced to disclose information that may identify a participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The Certificate cannot be used to resist a request for information from the United States government when it is used for evaluating federally funded study projects or for information that must be disclosed to meet the requirements of the Food and Drug Administration (FDA). A Certificate of Confidentiality does not prevent a participant or his/her family from voluntarily releasing information about the participant's involvement in this research. If an insurer, employer, or other person obtains a participant's or family's written consent to receive research information, then the researchers will not use the Certificate to withhold that information.

Information from plasma/serum/DNA analyses and clinical studies or medical records may be placed into a central data repository in the future, such as the National Center for Biotechnology Information (NCBI) repository. Data and samples will be de-identified before submission to this or any other central repository.

11.3 Potential Benefits

Treatment: For participants randomized to the pitavastatin treatment arm, potential benefits include lowering of cardiovascular risk due to improvement in lipid particle profile, other risk factors and arterial stiffness measures.

Test Findings: It is possible that the laboratory tests obtained for research purposes may disclose a cardiometabolic risk factor or diagnosis of importance to the participant's management (e.g. impaired fasting glucose, non-alcoholic fatty liver disease). Results will be provided to the participant's providers, and the participant's family will be informed about this information transfer.

Evaluation of cardiovascular risk status may provide valuable information that would not otherwise be available. If abnormalities are detected, this information may lead to early intervention measures designed to maximize developmental potential.

Biospecimens: Currently, there is no known direct benefit from the participation of the participant and family in the biorepository. However, we hope that sample donation will help investigators to learn more about the relationship between biomarkers and longer-term cardiovascular and metabolic outcomes. This information may help physicians provide better answers to families' questions regarding causes, risk, and recurrence risks. It may also provide clues to future interventions and/or treatments.

Indirect Benefit: There might be an indirect benefit from the awareness that study results may help to improve the care of children with similar problems in the future. Families may derive a sense of altruism, accomplishment, and contribution to furthering understanding of the problem through their participation.

11.4 Risk/Benefit Ratio and Importance of Information to Be Obtained

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The risk/benefit ratio of the study is favorable. The risk of adverse drug reactions is low, and most are relatively minor in nature and reversible with a decrease in the dose of study drug or cessation of therapy. The results of this study and the wealth of systematic data collected will make an important contribution to the management of CDO. All participants will receive lifestyle modification advice, and may benefit from the advice received as part of this study which is designed to deliver, at a minimum, usual care. In addition, the results of this study will make important contributions to the improvement of knowledge of the atherosclerotic process, the use of PWV, and ultimately in the improvement of treatment and prognosis.

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11.5 End of Study Return of Results to Families and their Healthcare Providers

At the end of the study, participants and/or parents will be sent a description of the overall study results in lay language. In this study, families will be given the treatment assignment and the contact information for the study PI and/or coordinator, in the event the family wishes to discuss the results or has questions. A separate notification will also be provided to the treating/referring physician (cardiologist, etc) describing overall study results.

12. STUDY LIMITATIONS

While this study will provide important evidence concerning the efficacy and safety of statin in treating CDO in adolescents, there will be some limitations. The trial will not determine the degree to which results from the trial in obese adolescents will be generalizable to non-obese adolescents with a similar lipid phenotype or those with type 1 or 2 diabetes. Given the trial is approximately 3 years long, it may not be of sufficient duration to fully assess both the benefits and risks of statin, particularly long term legacy benefits identified in adult trials. The trial will not provide evidence of a direct impact on CVD endpoints, and the impact on measures of early atherosclerosis may not have direct relevance to this endpoint.

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