STANFORD UNIVERSITY SCHOOL OF MEDICINE
DIVISION OF CARDIVASCULAR MEDICINE

Relationship Between Insulin Resistance and Statin Induced Type 2 Diabetes, and Integrative Personal Omics Profiling

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STUDY TITLE

Relationship Between Insulin Resistance and Statin Induced Type 2 Diabetes, and Integrative Personal Omics Profiling

ABBREVIATIONS

The following abbreviations will be used throughout this protocol:

Atherosclerotic Cardiovascular Disease: ASCVD
Type 2 Diabetes Mellitus: T2D
Metabolic syndrome: MetS
LDL cholesterol: LDL-C
HMG-coenzyme A reductase: HMGCR
High-sensitivity C-reactive protein hs-CRP
Oral Glucose Tolerance Test: OGTT
Insulin Suppression Test: IST
Graded Glucose Infusion Test: GGIT
Area under the curve: AUC
Glucose-stimulated Insulin Secretion Rate: ISR
Integrated Personal Omics Profiling: iPOP

Brief Description:

Background:
There is general agreement that statin-treatment of patients to lower plasma cholesterol levels can increase the incidence of type 2 diabetes mellitus (T2D) in some individuals[1-5]. The physiologic mechanism for the increased risk for T2D from statin treatment is unknown but could result from effects on insulin sensitivity or insulin secretion. This study will evaluate how the medication atorvastatin (trade name Lipitor) works in non-diabetic individuals in regards to its effect on insulin sensitivity and insulin secretion to help further understand the possible cause of the increased occurrence of T2D in people who are at risk for T2D. This research study will also examine what metabolic characteristics and variables (for example insulin resistance, high triglycerides, or both) will identify those people at highest risk of statin-induced T2D.

The goals of this study are to:

1) determine the effect of high-intensity atorvastatin (40 mg/day) for ~ 10 weeks on insulin sensitivity and insulin secretion (defined with gold standard methods) (**PRIMARY OUTCOMES**) as well as other glycemic traits (**SECONDARY OUTCOMES**);

2) compare a number of cardio-metabolic characteristics (e.g. weight, lipids) before, during, and after administration of atorvastatin;

3) determine if significant deterioration of insulin action and/or secretion following statin treatment will be confined to those with baseline insulin resistance (**PRE-SPECIFIED SUBGROUP ANALYSES**);

4) perform Personal Omics Profiling (iPOP) [6,7] before and after taking atorvastatin to examine treatment-associated changes in all baseline variables and to analyze not only previously-known drug efficacy but also untargeted drug efficacy (**EXPLORATORY ANALYSES**).

General approach:
This will be an open-label study to evaluate the diabetogenic effect of atorvastatin (40 mg/day for 10 weeks) on both insulin action and insulin secretion in nondiabetic individuals. To ensure we recruit individuals across a broad range of insulin sensitivity, we will target recruitment to enrich for those with combined increases in LDL-C and TG concentrations (see SIGNIFICANCE and RATIONALE). The experimental population will consist of ~75 apparently healthy, non-diabetic volunteers eligible for statin therapy but without pre-existing atherosclerotic cardiovascular disease. Following baseline assessments of co-primary outcome measures: insulin sensitivity (by insulin suppression test, IST) and insulin secretion (by graded glucose infusion test, GGIT), participants will be placed on a weight maintenance diet and treated with 40 mg/day of atorvastatin. All baseline measurements will be repeated ~10 weeks later with iPOP[8] measurements done at baseline, at weeks 2, 4, and 10 on atorvastatin, and at weeks 4 and 8 off atorvastatin.

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1. SIGNIFICANCE

Statins and the risk of T2D: Statin treatment is associated with an increase in incident T2D.[1-4 5] This adverse outcome seems to be a class effect, although there is some evidence for a dose dependent relationship. After analyses of large statin clinical trial data showed an increased risk of T2D associated with statin use, initial follow-up studies focused on the clinical impact of this risk. More recently, attention has been given to understanding why statins increase risk of T2D and the clinical characteristics that help identify those at increased risk.

Mechanism of statin-induced T2D: It is unclear whether statins increase the risk of T2D by decreasing insulin action, secretion, or both. Several manuscripts have been published that substantially increase understanding of the link between statin use and incident T2D. Swerdlow, et al.[2] based on evidence from genetic analysis and randomized trials, concluded that the increased risk of T2D noted with statins is at least "partially explained by HMG-coenzyme A reductase (HMGCR) inhibition." They also noted an association of weight gain with HMGCR variants in statin-treated patients, leading to the notion that decreases in insulin sensitivity contribute to statin-induced diabetes. In that context, Cederberg, at al.[9] have shown in a large prospective study (n=8749 men) that participants treated with statins (n=2142) had a 46% increase in incident T2D, associated with a 24% decrease in insulin sensitivity and a 12% decrease in insulin secretion assessed by surrogate measures.

Identifying those with at enhanced risk of statin-induced T2D: Studies of 3 randomized clinical trials with atorvastatin by David Waters' group [1,3,4] have demonstrated that "baseline fasting glucose, body mass index, hypertension, and fasting triglycerides were independent predictors of T2D." These abnormalities form a cluster, initially referred to as Syndrome X (or sometimes referred to as the Metabolic Syndrome, MetS) and attributed to insulin resistance.[10] Since insulin resistance is a predictor of developing T2D, it seems likely that the more insulin resistant the individuals are before treatment, the greater is their risk to for statin-induced T2D.

In that context, relatively little attention has been given to the role that metabolic heterogeneity in patients with elevated LDL-C concentrations might play in statin-induced T2D. Specifically, subjects with elevated LDL-C concentrations, whose plasma triglyceride (TG) concentrations are also elevated, are insulin resistant, hyperinsulinemic, and glucose intolerant as compared to those with isolated LDL-C levels. As such, this subset of patients with elevated LDL-C concentrations can be viewed as being at a "tipping point," and any adverse effect of statins on insulin action and/or secretion, irrespective of how mediated, places them at enhanced risk to develop statin-induced diabetes. Indeed, we have shown (Kohli et al)[1] that patients with both insulin resistance (as estimated by high TGs) and prediabetes are at particularly high risk of statin-induced T2D.

While there are other possible approaches to identify individuals most at risk to develop T2D when treated with statins, e.g., a diagnosis of the metabolic syndrome (MetS), a comparison of 291 apparently healthy individuals in our database, grouped together on the basis of having the MetS vs. a plasma TG concentration ≥ 150 mg/dL, revealed comparable cardio-metabolic risk profiles. Given this information, it seemed reasonable to continue evaluating the ability of a plasma TG concentration of ≥ 150 mg/dL as a surrogate for insulin resistance to identify those most at risk to develop T2D when taking statins.

We seek to combine our understanding of the phenotypic heterogeneity of individuals with elevated LDL-C concentrations with quantification of insulin action and secretion with gold standard methods to address 2 important unanswered questions: Do statins primarily affect insulin resistance or insulin secretion?; Are there subsets of individuals at highest risk of statin-induced T2D?

2. RATIONALE, HYPOTHESIS AND OUTCOME MEASURES

Rationale:

T2D develops when insulin resistant individuals cannot maintain the degree of compensatory hyperinsulinemia needed to maintain normal glucose tolerance. However, significant fundamental questions remain. For example, what is the cellular/molecular link between statin treatment and changes in insulin action and secretion?

This proposal is based on the premise that studying the effect of statins on insulin action and insulin secretion using “gold standard” methods will help determine if statins adversely affect the risk of T2D by increasing insulin resistance or decreasing insulin secretion. We use the insulin suppression test (with the read-out of steady-state plasma glucose, SSPG) to ascertain insulin sensitivity and the graded glucose infusion test (with the readout of insulin secretion rate, ISR) to ascertain insulin secretion both before and after statin treatment in non-diabetic individuals.

We hypothesize that treatment with atorvastatin 40 mg/day for approximately 10 weeks will impair insulin sensitivity and/or insulin secretion and that this effect may be exacerbated in those with underlying insulin resistance. Thus, we plan to look at the effect of atorvastatin not only in all participants but also in subsets of individuals with baseline insulin resistance (which will be enriched for by recruiting volunteers with elevated plasma TG levels (≥150 mg/dL) at baseline. The rationale for this is that plasma TGs are a surrogate measure for insulin resistance with a modest correlation with the direct measure of insulin resistance (steady-state plasma glucose) measured by the insulin suppression test. Clinically, subjects with elevated TGs prior to statin treatment would have substantial clinical benefit from statins, and one of investigators' secondary goals is to demonstrate that a simple measurement of plasma TG concentration (as a surrogate for insulin resistance) can help identify those most at risk of statin induced derangements in glycemic control. Consequently, we propose to enroll nondiabetic volunteers at high-risk for T2D, free of known atherosclerotic cardiovascular disease (ASCVD), not receiving statins, eligible for statin therapy according to ACC/AHA (American College for Cardiology/American Heart Association) 2013 guidelines.[11] We will also target recruitment efforts to enrich for subjects with plasma TG concentration ≥ 150 mg/dL to ensure that we enroll subjects across the range of insulin sensitivity.

Hypothesis: We hypothesize that high intensity atorvastatin treatment for approximately 10 weeks will impair insulin sensitivity and/or insulin secretion and that this effect may be exacerbated in those with underlying insulin resistance.

Primary Outcome Measures:

- Change in insulin sensitivity
    - Insulin Sensitivity: Steady-state plasma glucose concentration (mg/dL) measured during the insulin suppression test (IST)
    - Time Frame: Change from baseline to 9 -10 weeks in insulin sensitivity
- Change in insulin secretion
    - Insulin secretion: Insulin secretion rate AUC (pmol/min x 4 h) measured during the graded glucose infusion test (GGIT)
    - Time Frame: Change from baseline to 9 -10 weeks in insulin secretion

Secondary Outcome Measures:

- Change in fasting plasma glucose and fasting plasma insulin
    - Fasting plasma glucose and insulin
    - Time Frame: Change from baseline to 8 - 10 weeks in fasting plasma glucose and insulin

- Change in OGTT glucose AUC and insulin AUC
    - OGTT glucose AUC (mg/dL x 2 h) and OGTT insulin AUC (mU/L x 2h)
    - Time Frame: Change from baseline to 8 weeks in OGTT glucose AUC and insulin AUC

Prespecified Subgroup Analyses

- Determine if significant deterioration of insulin action and/or secretion following statin treatment will be confined to those with baseline insulin resistance.

Exploratory analyses:

- Personal Omics Profiling Personalized medicine is expected to benefit from the combination of genomic information with the global monitoring of molecular components and physiological states[6,7]. To further extend Dr. Snyder's previous research of integrated Personal Omics Profiling (iPOP), which monitors genomic, transcriptomic, proteomic, metabolomic, and autoantibodyomic information over time, the investigators propose to analyze iPOP of apparently healthy volunteers with dyslipidemia longitudinally before, during, and after taking atorvastatin. In this pilot substudy, by performing unprecedented depth of omics analysis, both previously known and untargeted efficacies of atorvastatin will be analyzed. Overall, the endeavor will improve our understanding of how to perform a personal omics profile when taking statins and help us develop recommendations for better use of statins.

3. STUDY DESIGN

Sample Size

- We aim to recruit and retain 75 total participants in this study.

Study Population (see Appendix A for detailed inclusion and exclusion criteria**)**

- Healthy adults 30 - 70 years old.
- BMI: 20 - 37 kg/m^2.
- Persons without diabetes as defined by having fasting plasma glucose < 126 mg/dL and not taking glucose lowering medications.
- Individuals eligible for statin therapy for primary prevention of ASCVD based on LDL-C ≥ 130 mg/dL, > 5% ASCVD risk over 10 years, or hs-CRP ≥ 2.0 mg/L.

Study Location

- Research Participants will be seen and have all study procedures at: Clinical and Translational Research Unit (CTRU) at 800 Welch Road, Palo Alto, CA 94304.

Duration

- We anticipate that the entire study will take 4 - 5 years through the end of data analysis. Each eligible candidate who voluntarily consents to participate in the study will be active in the study for a total of 5 months from screening to the end of their last visit.
- Month: 1
    - Prepare IRB/Human Subjects materials (2 months total).
- Month: 2-3
    - Prepare all recruitment materials.
    - Prepare all study protocols and materials.
    - Finish IRB protocol approval.
- Months: 4
    - Begin study recruitment.
    - Each patient will be in study for approximately 5 months.
    - Goals is 15 - 20 participant enrollment per year.
- Months: 5-12
    - Begin screening and enrolling study participants. Each participant will complete the ~ 5 -month protocol of the study.
    - Data clean-up on an ongoing basis.
- Month: 30
    - Send out OGTT, IST and GGIT blood samples for the first 30 participants for measurement of glucose, insulin, and C-peptide.
- Month: 48
    - Last participant completes the study.
- Months: 49-50
    - Finish record update for remaining blood samples and ship out OGTT, IST, and GGIT samples for measurement of glucose, insulin, and C-peptide.
    - Start rechecking and clean-up of data and begin data analysis.
- Month: 52
    - Receive the lab analysis results.
    - Continue with study data analysis and prepare manuscript.

4. STUDY PROCEDURES

Recruitment

Preliminary recruitment strategies will include:
Volunteers will be recruited from the San Francisco Bay Area through advertisements in newspapers, posted flyers, and the social networking site NextDoor as well as from the Preventive Cardiology Clinics at Stanford Health Care. Our goal is to ensure recruitment across a broad range of insulin sensitivity. Prior work from our group and others has shown that high plasma TG concentrations are associated with increased insulin resistance as assessed by reference measures. Therefore, we will target advertisements to enrich for individuals with high TG levels (³ 150 mg/dL) as a surrogate for increased insulin resistance.

Participant Visits and Procedures

Potential participants will be screened initially when they call or email in response to recruitment ads, or a letter from their MD, describing the study as follows:

Preliminary intake will occur over the phone. Potential participants will be asked their height and weight, if they have any major health problems, specifically T2D, cardiovascular disease or kidney disease, and a list of their current medications. We will also ask potential participants if they know their lipid profiles, if they have been on statins in the past, and if they are currently on a statin or other lipid lowering medication. Demographic information (name, date of birth and address) is required to establish screening appointment in CTRU and a medical record number.

If they meet preliminary inclusion/exclusion criteria, an appointment will be made for a screening visit. The consent will be emailed prior to the visit to allow them to review before the screening visit.

Name, height, weight, and phone number are requested on research line to call back appropriate people. That information is written down and is discarded after ~ 6 months. Any other information we obtain over the phone is also discarded in confidential bin.

Visit 1 Screening visit.

For the first visit, potential participants will be invited to the Stanford CTRU for screening. Participants must fast (excluding water) for 8 -10 hours prior to the appointment. They will be given a copy of the consent to first read on their own. Once they are ready, a member of the research group will review and explain the study to the patient and answer any questions. If they agree to participate, they will sign the consent form, which will be witnessed and signed by the researcher, and a copy of the consent form will be given the participant.

Once consent is signed, a detailed medical history will be taken to further determine eligibility criteria. Participants will undergo measurement of height, weight, blood pressure, and calculation of BMI. Blood will be drawn and hematocrit and fasting plasma glucose will be analyzed in the CTRU lab and lipid concentrations, hs-CRP, liver function tests and serum creatinine will be determined by standard laboratory techniques in Stanford Health Care (SHC) laboratory.

If there are no contraindications to participating based on further data obtained at the screening visit including laboratory results noted above, then they will proceed to be scheduled for an oral glucose tolerance test.

Visit 2:

Oral Glucose Tolerance Test (OGTT): This test will take approximately 3 hours.
The OGTT involves a series of blood samples taken before and during 120 minutes after drinking Dexola/Glucola containing 75-gram glucose. This test will be done before and after

taking the study medication atorvastatin 40/day. Participants will have fasted for 12 hours from the previous evening. One intravenous (IV) catheter will be inserted in their arm for the purpose of drawing blood samples to determine blood glucose level during this procedure at zero, 30 min, 60 min, 90 min, and 120 min. The total amount of blood taken for this test will be 33.5 ml. The purpose of this procedure is to define:

i) Glycemic status: Participants will be classified as having normal glucose tolerance (NGT), isolated impaired fasting glucose (IFG), isolated impaired glucose tolerance (IGT), or combined IFG/IGT[12]. In addition, the total integrated plasma glucose response during the OGTT will be calculated by the trapezoidal method (Glucose-AUC).

ii) β-cell function: Calculations of the Insulin Secretion- Sensitivity Index-2 (ISSI-2) will be used to quantify insulin secretory function [13], if for some reason a graded glucose infusion study is not done. The ISSI-2 is a validated OGTT- derived method to measure of β-cell function, analogous to the disposition index obtained from the intravenous glucose tolerance test [14]. It is calculated by multiplying the insulin secretory response during the OGTT (Insulin-AUC/Glucose-AUC) by insulin resistance (Matsuda index).

The fasting and 2-hour glucose results will be discussed with the study participant and a copy of the results will be given to them.

Visit 3.

Graded Glucose Infusion, GGIT [15,16]: This test will take approximately 6 hours.
This test is designed to assess the ability of the pancreas to produce insulin in response to a graded glucose infusion. During this test, subjects will have two small IV catheters placed, one in each arm. One IV will be used for drawing samples and the other for the infusion of glucose. During the GGIT, continuous intravenous infusions of glucose will be given at progressively increasing rates: 1, 2, 3, 4, 5, 6, and 8 kg/min in six infusion periods of 40-min duration. Blood samples will be collected for measurements of glucose, insulin, and C-peptide concentrations at fasting and at 30 min and 40 min into each infusion period. The two values during the last 10 min of each infusion period will be averaged. The amount of blood taken for this test will 47.5 ml.

Visit 4.

Insulin Suppression test, IST [17,18]: This test will take approximately 6 hours.
This test is designed to determine whole body insulin sensitivity. Following an overnight fast, subjects will have an IV placed in each arm. One for collection of blood and the other for infusion with octreotide (0.27 μg/m^2/min), insulin (32 mU/m^2/min), and glucose (267 mg/m^2/min) for 180 minutes. During the test, endogenous insulin is suppressed and all individuals are given the same concentration of insulin, based on their body surface area. Blood is drawn every 30 minutes for 150 minutes and then at 10-minute intervals from 150 to 180 minutes of the infusion to measure plasma glucose and insulin concentrations. The mean of the last four values is used as the steady-state plasma insulin (SSPI) and glucose (SSPG) concentrations for each individual. As SSPI concentrations are similar in all subjects during the IST, the SSPG concentration provides a direct measure of the ability of insulin to mediate disposal of an infused glucose load; the higher the SSPG concentration, the more insulin resistant the individual. Labs to check kidney and liver function plus a lipid panel and a urine pregnancy test (if appropriate) will be done at this visit. Blood drawn or IST will be 58.5 ml and 5 ml for SHC labs.

Labs for iPOP will be drawn at this time and additional samples will be obtained for transcriptome, microbiome, metabolome, and proteome analysis in blood; nasal, tongue, skin surface swabs; urine; and stool. Total blood draw is 54 ml for omics. Participants will fill out questionnaires at the time of iPOP samples. The questionnaires will be on physical activity,

stress and diet. Laboratory measures will include complete blood count, comprehensive metabolic profile, hemoglobin A1c, insulin and IgM levels.

- **The study drug, atorvastatin 40 mg will be given to study participants, once all labs have been reviewed and participant qualifies.**

Visit 5-7
Visits will be every 2 weeks for a total of 10 weeks on study medication (statin). Participants will be assessed for any side effects or adverse events (AE) on the statin. Adherence to study medication will be assessed at each visit.
At each visit, weight and vital signs will be done as well as iPOP lab testing as described above except no iPOP will be done at visit 6.

Visit 8:
Weight, vital signs, and OGTT described above.

Visit 9:
Repeat GGIT as described above.

Visit 10:
Repeat IST and iPOP lab testing and samples as described above.
At the end of this visit, the statin will be stopped and the study participant would be scheduled for 4- and 8-week follow up visits.

Visit 11:
One month off statin study visit – weight, vital signs, and iPOP laboratory testing and samples will be done as described above.

Visit 12:
Last study visit – weight, vital signs, and iPOP laboratory testing and samples will be done as described above.
Participants will be asked to fill out questionnaire about her/his physical activity status, food and eating habits, and stress at the time of each iPOP.

We will not be providing any iPOP results to study participants per protocol.

Study Medication Management of Atorvastatin 40 mg
Atorvastatin: The most commonly reported adverse reactions (incidence ≥ 2%) in patients treated with atorvastatin 40 mg in placebo-controlled trials regardless of causality were: nasopharyngitis-7%, arthralgia-10.6%, diarrhea-14.1 %, pain in extremity- 9.3%, and urinary tract infection-8.0%.
WARNINGS AND PRECAUTIONS: Skeletal Muscle
Rare cases of rhabdomyolysis with acute renal failure secondary to myoglobinuria have been reported with LIPITOR and with other drugs in this class. A history of renal (kidney) impairment may be a risk factor for the development of rhabdomyolysis. People with kidney disease will not qualify for this study.

Lipitor, like other statins, occasionally causes myopathy, defined as muscle aches or muscle weakness in conjunction with increases in creatine phosphokinase (CPK) (enzyme measuring muscle breakdown) of values >10 times of upper limits of normal-ULN. The use of higher doses of atorvastatin with certain drugs such as cyclosporine and strong CYP3A4 inhibitors (e.g., clarithromycin, itraconazole, and HIV protease inhibitors) increases the risk of myopathy/rhabdomyolysis. We will only be using 40 mg not the highest dose.

Myopathy should be considered in any patient with diffuse myalgias, muscle tenderness or weakness, and/or marked elevation of CPK. Patients should be advised to report promptly unexplained muscle pain, tenderness, or weakness, particularly if accompanied by malaise or fever or if muscle signs and symptoms persist after discontinuing Lipitor. Lipitor therapy should be discontinued if markedly elevated CPK levels occur or myopathy is diagnosed or suspected. There have been rare reports of immune-mediated necrotizing myopathy (IMNM), an autoimmune myopathy, associated with statin use. IMNM is characterized by: proximal muscle weakness and elevated serum creatine kinase, which persist despite discontinuation of statin treatment; muscle biopsy showing necrotizing myopathy without significant inflammation; and improvement with immunosuppressive agents.

The risk of myopathy during treatment with drugs in this class is increased with concurrent administration of cyclosporine, fibric acid derivatives, erythromycin, clarithromycin, certain medications for treating HIV or hepatitis C, protease inhibitor telaprevir, niacin, or azole antifungals. Creatine phosphokinase (CPK) determinations may be considered in such situations, but there is no assurance that such monitoring will prevent the occurrence of severe myopathy. Patients with HIV or Hepatitis C will not qualify for this study

At each visit, we assessed for any sided effects or adverse effects (AE). A lipid panel with calculated LDL and comprehensive metabolic profile will be drawn (for iPOP) at weeks 2, 4, and 10 while on the study drug. If there are any side effects or myalgia, a creatinine kinase will be checked, study medication will be stopped until symptoms are resolved and then restarted. If symptoms return, with or without laboratory abnormalities, the atorvastatin dose will be decreased to 20 mg/day.

5. STATISTICAL CONSIDERATIONS

Based on our prior work[19], we calculated that with 60 subjects, we would be able to detect an 8% change in SSPG concentration and an 8% change in ISR_{AUC} after atorvastatin therapy with 80% power and two-side significance level of 5% using a paired samples t test. Thus, we estimated needing to enroll 75 subjects with an anticipated a dropout rate of 20%.

Summary statistics will be reported as median (interquartile range) or number (percent) of participants unless otherwise specified. Shapiro-Wilk tests will be used to assess normality of data, and variables that are not normally distributed will be log-transformed. Percent changes in variables will be calculated by the formula: [(end-of study value) - (baseline value) / baseline value] x 100. Paired samples t tests will be used to compare baseline and end-of-study means. One sample t tests will be employed to evaluate whether percent changes in variables are significantly different from zero (no change). Pearson correlation coefficients will be calculated to determine the strength of association between variables of interest. Prespecified subgroup analyses will be carried out by stratifying for insulin resistant versus insulin sensitive subjects. The SSPG concentration median will be used to define subjects as being insulin resistant or insulin sensitive. Insulin resistant and insulin sensitive group means will be compared by independent sample t tests and proportions by chi-square tests or Fisher's exact tests. Statistical analyses will be performed by using statistical software IBM SPSS version 26.0.

APPENDIX

A. Inclusion/Exclusion Criteria

Eligibility Criteria

Inclusion Criteria:

- Healthy adults 30 - 70 years old.
- BMI: 20 - 37 kg/m^2.
- Persons without diabetes as defined by having fasting plasma glucose < 126 mg/dL and not taking glucose lowering medications.
- Individuals eligible for statin therapy for primary prevention of ASCVD based on LDL-C ≥ 130 mg/dL, > 5% ASCVD risk over 10 years, or hs-CRP ≥ 2.0 mg/L.

Exclusion Criteria:

- Younger than 30 or older than 70 years.
- Persons with any significant co-morbidities, such as diabetes (fasting glucose ≥ 126 mg/dL or use of glucose lowering medications), active coronary artery disease or heart failure, accelerated or malignant hypertension, kidney disease (creatinine ≥ 1.5 mg/dL), liver disease (alanine aminotransferase > 2 times upper limit of normal), or severe anemia (hematocrit < 30%).
- Individuals taking any medications for weight loss or known to influence insulin sensitivity.
- Pregnant or lactating.
- Women unwilling to use an effective birth control method.
- History of statin intolerance.

B. Participant Expectations Schedule Example

Schedule of Study Visits

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12
	Week -3	Week -2	Week -1	Week 0	Week 2	Week 4	Week 6	Week 8	Week 9	Week 10	Week 14	Week 18
Screening	X											
Lipids	X			X						X	X	X
OGTT		X						X				
GGIT			X						X			
IST				X						X		
Follow-up					X	X	X				X	X
Atorvastatin 40 mg				Start						Stop		

Individuals on a stain at the screening visit will undergo a 4-week statin washout before the baseline metabolic tests. GGIT indicates graded glucose infusion test; IST, insulin suppression test; and OGTT, oral glucose tolerance test.

C. Outcome Measurement Methods

Glucose, Insulin, C-Peptide (Blood): Insulin and C-peptide will be measured by radioimmunoassay (Millipore, St. Charles, MO). These will be analyzed by the Core Laboratory for Clinical Studies at the Washington University School of Medicine in St Louis, MO. Homeostasis model assessment of insulin resistance (HOMA-IR) will be calculated by the following formula: (fasting insulin (mU/L) x fasting glucose (mmol/L))/22.5.

Lipids (Blood) LDL-C will be calculated according to Friedewald (1972).

Anthropometrics. Height will be measured to the nearest mm on a standard wall-mounted stadiometer. Body weight will be measured to the nearest 0.1 kg, in duplicate, on a calibrated clinical scale. Body mass index (BMI) will be calculated as weight in kg divided by height in m^2, and waist circumference will be measured at the midpoint between upper iliac crest and lower end of the rib cage in mid respiration while subjects are standing. Blood pressure will be measured by using an automated blood pressure monitor with appropriately sized arm cuff. Before blood pressure measurement, participants will sit quietly in a chair for 5 minutes with arm supported at the heart level.
Weight and vital signs will be measured at every visit, and waist circumference will be done at baseline and post study IST.

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