

University of Pennsylvania

INvestigation of the Gut microbiomE on STatin response (INGEST)

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| Principal Investigator     | Sony Tuteja, PharmD, MS<br>Department of Medicine<br>Perelman School of Medicine at the University of Pennsylvania<br>3400 Civic Center Blvd<br>Smilow Center for Translational Research, 11-143<br>Philadelphia, PA, 19104<br>215-573-7834 (phone)<br>215-573-8606 (fax)<br><a href="mailto:sonyt@pennmedicine.upenn.edu">sonyt@pennmedicine.upenn.edu</a> |
| Co-Investigators:          | Archna Bajaj, MD<br>Daniel Rader, MD<br>Gary Wu, MD, PhD                                                                                                                                                                                                                                                                                                    |
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**List of Abbreviations**

|                  |                                     |
|------------------|-------------------------------------|
| AUC              | Area under the curve                |
| CHPS             | Center for Human Phenomic Science   |
| CVD              | Cardiovascular disease              |
| C <sub>max</sub> | Concentration at peak               |
| FDA              | Food and Drug Administration        |
| FGF19            | Fibroblast growth factor 19         |
| FXR              | Farnesoid X receptor                |
| IDS              | Investigational drug service        |
| LDL-C            | Low density lipoprotein cholesterol |
| MAT              | Mean absorption time                |
| PK               | Pharmacokinetic                     |
| OTU              | Operational taxonomic units         |
| T <sub>max</sub> | Time to peak concentration          |

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## Study Summary

|                                 |                                                                                                                                                                                                                                                                                                                                                             |
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| Title                           | <u>I</u> nvestigation of the <u>G</u> ut microbiom <u>E</u> on <u>S</u> Tatin response                                                                                                                                                                                                                                                                      |
| Short title                     | INGEST                                                                                                                                                                                                                                                                                                                                                      |
| Protocol number                 | 832874                                                                                                                                                                                                                                                                                                                                                      |
| Phase                           | Not applicable                                                                                                                                                                                                                                                                                                                                              |
| Methodology                     | Randomized, placebo-controlled study in healthy subjects                                                                                                                                                                                                                                                                                                    |
| Study duration                  | 2 years                                                                                                                                                                                                                                                                                                                                                     |
| Study center                    | Single center                                                                                                                                                                                                                                                                                                                                               |
| Objectives                      | <p>This study is designed to determine the relationship between the gut microbiome composition, fecal bile acid levels, serum FGF19 levels and LDL-C lowering response to rosuvastatin.</p> <p>A subset of participants will participate in an optional rosuvastatin pharmacokinetic evaluation.</p>                                                        |
| Number of subjects              | 100                                                                                                                                                                                                                                                                                                                                                         |
| Main inclusion criteria         | Healthy participants between the ages of 18 and 65 in overall good health, , who have not taken antibiotics in the preceding 3 months.                                                                                                                                                                                                                      |
| Study product                   | Rosuvastatin 20 mg daily                                                                                                                                                                                                                                                                                                                                    |
| Duration of administration      | 8 weeks                                                                                                                                                                                                                                                                                                                                                     |
| Reference therapy               | Placebo                                                                                                                                                                                                                                                                                                                                                     |
| Statistical methodology         | Comparison of the bacterial composition, fecal bile acid concentrations, serum FGF19 between statin and placebo treated groups from baseline to 8 weeks. The association between changes in microbial composition with clinical characteristics (sex, race, age, BMI, diet) and outcome (the change in LDL-C) will be assessed using multivariate modeling. |
| Safety evaluations              | Expected and unexpected rosuvastatin related adverse events will be monitored by the study team.                                                                                                                                                                                                                                                            |
| Data and safety monitoring plan | An independent review of the safety data will be performed by a data and safety monitoring board.                                                                                                                                                                                                                                                           |

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## Background and Study Rationale

This study will be conducted in full accordance all applicable University of Pennsylvania Research Policies and Procedures and all applicable Federal and state laws and regulations including [as applicable include the following regulations as they apply 45 CFR 46, 21 CFR Parts 50, 54, 56, 312, 314 and 812 and Good Clinical Practice: Consolidated Guidelines approved by the International Conference on Harmonization (ICH). All episodes of noncompliance will be documented.

## 1. Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide, despite the availability of evidence-based strategies such as high-dose statins, which can effectively reduce plasma low-density lipoprotein cholesterol (LDL-C) concentrations by up to 50% and cardiovascular events by 30%. However, some susceptible individuals continue to experience cardiac events (referred to as residual risk) even while on aggressive statin therapy, and overall there is a great deal of variability and inadequate response to the LDL-C lowering effects of statins. Together, these observations suggest that an improved understanding of the factors contributing to both pharmacological variability to statin therapy as well as to the residual risk will enhance our efforts in decreasing CVD related morbidity and mortality.

Accumulating evidence suggests that the intestinal microbiota impacts the development of CVD<sup>1, 2</sup>, host plasma lipid levels<sup>3, 4</sup>, metabolism of xenobiotics<sup>5-8</sup>, and has shown to be a contributing factor in drug variability.<sup>8</sup> Previous animal studies have demonstrated alterations in gut microbiome composition secondary to statin drugs.<sup>9-11</sup> An observational study in human subjects showed that short-term treatment with rosuvastatin altered the gut microbiome diversity and taxa in association to the LDL-lowering effects; however the mechanism of alteration was not investigated.<sup>12</sup> Bile acids, synthesized from cholesterol, are highly modified by the gut microbiome, and play a role in cholesterol and bile acid homeostasis via signaling through the bile acid nuclear receptor farnesoid X receptor (FXR)-fibroblast growth factor 19 (FGF19) enterohepatic pathway. While there is an established link between composition and functionality of the gut microbiome and host lipid metabolism, there remains a knowledge gap in our understanding of how perturbations in the gut microbiome by statins influence bile acid metabolism and subsequently the variations in plasma LDL-C.

### 1.1 Background and relevant literature

Cardiovascular disease (CVD) remains the leading cause of death in men and women the United States and Worldwide.<sup>13, 14</sup> Elevated plasma low-density lipoprotein cholesterol (LDL-C) is causally linked to coronary disease.<sup>15</sup> The American Heart Association recommends high-intensity statins for patients at high risk for cardiovascular events.<sup>16</sup> The recommended statins are expected to reduce LDL-C by at least 50%, however treatment response is individual and varied, with a high proportion of individuals exhibiting a suboptimal outcomes (<30% reduction in LDL-C). Strikingly, even after aggressive LDL-lowering therapy there remains a high rate of cardiovascular events (i.e. residual risk) in susceptible individuals.<sup>17</sup> Clinical trials with fish oils,<sup>18</sup> fibrates<sup>19</sup> and niacin<sup>20, 21</sup> have shown mixed results in reducing residual risk. A recent meta-analyses of statin interventional trials encompassing 32,258 patients from 37 trials, showed the standard deviation of LDL-C reduction for all statins and doses ranged from 12.8 to 17.9% and the percentage of patients experiencing suboptimal responses ranged from 5.3 to 53.3%.<sup>22</sup> This variability was not related to specific statin or dose. In the Jupiter study<sup>23</sup>, where healthy subjects with a median baseline LDL-C of 108 mg/dL (IQR 94-119 mg/dL) received rosuvastatin

20 mg, 46% experienced a LDL-C reduction of  $\geq 50\%$ , 43% experienced a LDL-C reduction between 0% and 50%, and 11% experienced no reduction or an increase in LDL-C compared with baseline. Genome-wide association (GWA) analysis has been applied to understanding the genetic basis of the variation in LDL-C lowering response, but has identified only a handful of variants.<sup>24-27</sup> In the most recent pharmacogenomic meta-analysis of LDL response to statins in 40,000 individuals, only four loci reached genome-wide significance (*APOE*, *LPA*, *SORT1*, *SLCO1B1*), and together they explained only  $\sim 5\%$  of the variation in LDL-C response to statin treatment. Genetic variation only explains a small fraction of the variation in statin response, and a deeper understanding of the additional factors contributing to pharmacological variability in LDL-lowering response to statin treatment will be important in reducing CVD risk.

The human body is colonized by trillions of microorganisms, collectively known as the microbiome, which possess hundreds of times as many genes as coded in the human genome.<sup>28</sup> Advances in high throughput sequencing technology such as 16S amplicon sequencing or shotgun metagenomics has allowed for the identification of microorganisms without the need for culturing.<sup>29, 30</sup> The gut microbiome is a complex community that provides important metabolic functions;<sup>28</sup> and alterations in the gut microbiome are associated with pathogenesis of several human diseases such as obesity<sup>31</sup>, diabetes<sup>32</sup> and atherosclerosis.<sup>1, 2</sup> The intestinal microbiota also regulate host lipid metabolism.<sup>3, 4, 33, 34</sup> The gut microbiome has recently been shown to explain a large proportion of variance in plasma lipids, independent of host genetics.<sup>4</sup> In a cross-sectional study, the microbiota explained 6% of the variance in TG and 4% in HDL-C; this is similar in magnitude to that explained by host genetics.<sup>4</sup> Prospective, interventional studies are required to further validate these findings. It is unknown if statins could influence cardiovascular risk in part through their effects on the gut microbiome (Figure 1).

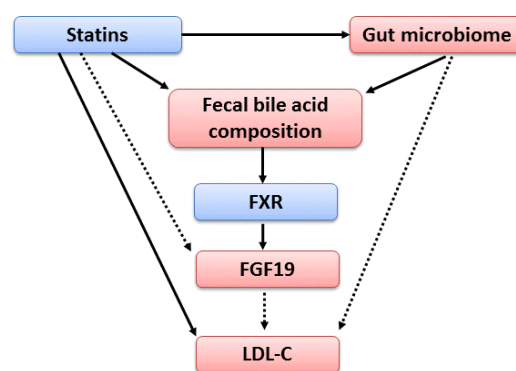


**Figure 1. Framework for investigating how variability in statin response is mediated through the gut microbiome.** Solid lines represent known effects; Dashed lines represents unknown effects. Components in the pink boxes will be measured as part of the study.

The gut microbiome plays an important role in the metabolism of xenobiotics and contributes to the variation in drug response.<sup>5-7, 35, 36</sup> Greater than 50 drugs display evidence for metabolism by the gut microbiome.<sup>8</sup> For example, *eggerthella lenta* converts the cardiac drug digoxin into the inactive microbial metabolite dihydrodigoxin.<sup>37</sup> Atorvastatin, simvastatin and rosuvastatin, three of the most commonly prescribed statin medications, also display evidence for modulation by the gut microbiome. Treatment of rats fed a high-fat diet with atorvastatin showed significant alterations in gut microbial communities towards species similar to healthy control rats fed a normal diet.<sup>10</sup> In a study involving unbiased plasma metabolomic profiling in human subjects, three secondary, bacterial-derived bile acids predicted the magnitude of simvastatin induced LDL-C lowering.<sup>35</sup> Pre-treatment concentrations of chenodeoxycholic acid and deoxycholic acid were correlated with on-treatment plasma simvastatin concentrations.<sup>35</sup> Treatment of mice with rosuvastatin modulated the composition of the bacterial species, and reduced hepatic expression of the *CYP27a1*,<sup>9</sup> which encodes an enzyme regulating the conversion of 7 $\alpha$ -hydroxycholesterol to chenodeoxycholic acid, a primary bile acid.<sup>38-40</sup> Rosuvastatin, by inducing gene expression changes, altered the concentrations of bile acids which contribute to the composition of gut microbiota. Most recently, a study in human subjects with hyperlipidemia showed that 4-8 weeks of rosuvastatin 10 mg significantly altered the gut microbiome and the abundance of specific bacterial taxa which was correlated to the LDL-C

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lowering response of the drug.<sup>12</sup> To our knowledge, no well controlled studies have yet explored the contribution of the gut microbiome to the LDL-C lowering response of statin therapies.

### 1.1.1 Role of Bile Acid Metabolites

The gut microbiota can impact host function through the generations of bioactive metabolites such as peptides, antibiotics, amino acids, and bile acids that can mediate host receptor activation, signaling and immunomodulatory effects.<sup>41, 42</sup> For example, trimethylamine-N-oxide (TMAO), a metabolite derived from dietary choline and carnitine by the actions of the gut microbiome was initially discovered by unbiased liquid chromatography-mass spectrometry (LC/MS) screen from plasma of patients with CHD.<sup>43</sup> In several subsequent studies TMAO was confirmed to be pro-atherogenic, pro-thrombotic and a causal contributor to CHD risk.<sup>44-46</sup> Small molecules inhibiting the microbial production of the TMAO precursor, trimethylamine, are currently being pursued.<sup>47</sup> Gut microbiota have been linked with lipid metabolism through their role in bile acid metabolism.<sup>39, 48-50</sup> Cholesterol is converted into the primary bile acids, cholic acid and chenodeoxycholic acid in the liver, and excreted in the bile after conjugation to glycine or taurine to facilitate dietary lipid absorption.<sup>49</sup> Bile salts excreted into the intestine are deconjugated back to primary bile acids by bacterial *bile salt hydrolase* (*bsh*) enzymes.<sup>49</sup> Primary bile acids are converted to secondary bile acids, lithocholic acid and deoxycholic acid, by anaerobic gut bacteria involving dihydroxylation via the expression of genes in the *bile acid inducible operons* (BAI), which increases bile acid hydrophobicity and facilitates reabsorption in the colon.<sup>51</sup> Gut bacteria modify the composition of bile acids in the intestinal lumen, thereby impacting the nature of the circulating bile acid pool, while conversely, bile acids in the intestine impact the survival and growth of bacteria.<sup>51</sup> Model-dependent differences, likely due in part to differences in species-dependent bile acid metabolism, have confounded the interpretation of this bidirectional interplay between the gut microbiome and bile acids, and importantly, **a significant knowledge gap exists with respect to the causal impact of statin drugs on the microbiome and bile acid homeostasis,**<sup>11, 52-54</sup> In a recent study in mice, atorvastatin decreased the amounts of secondary bile acids in the small and large intestine.<sup>11</sup> In a randomized placebo controlled clinical trial of 127 participants, treatment with *Lactobacillus reuteri*, a bacteria with elevated *bsh* activity, was shown to significantly reduce LDL-C levels.<sup>55</sup> In addition, individual changes in LDL-C were inversely correlated with circulating bile acids. Bile salt hydrolase activity has been previously characterized in *Firmicutes*,<sup>56</sup> a species previously associated with LDL-C in response to rosuvastatin.<sup>12</sup>

Bile acids can also modulate lipid metabolism through activation of the nuclear receptor FXR (farnesoid-X receptor) located throughout the small intestine and colon.<sup>50, 57</sup> Individual bile acids have varying ability to activate FXR (chenodeoxycholic acid> cholic acid>deoxycholic acid >lithocholic acid),<sup>58</sup> and perturbations of intestinal bacteria involved in bile acid metabolism by statins will affect the ratio of primary and secondary bile acids and its downstream signaling response. Since microbial enzymes have the potential to create dozens of bile acid metabolites, each with differing potential to activate host receptors, further investigation in humans into how gut-derived metabolites may interface with lipid metabolism is warranted.

### 1.1.2 FGF19 and LDL-C

Among the genes induced by FXR activation is fibroblast growth factor 19 (FGF19). Bile acids activate FXR to secrete FGF19 into the portal circulation where it has two main effects: 1) it travels to the liver to inhibit bile acid synthesis by repressing transcription of cholesterol 7 $\alpha$ -hydroxylase (CYP7A1), which encodes the first and rate-limiting step in the bile acid synthetic pathway; and 2) causes the gall bladder to fill with bile.<sup>59</sup> Circulating FGF19 levels reflect ileal exposure to bile acids.<sup>60, 61</sup> Circulating serum levels of FGF19 in healthy subjects vary widely (7.4 fold) with a large inter-individual variation.<sup>61</sup> Treatment with the bile acid resin,

cholestyramine, strongly reduces FGF19 levels, whereas treatment with the primary bile acid chenodeoxycholic acid increases FGF19.<sup>62</sup> The FXR-FGF19 pathway is currently being studied in the treatment of diabetes, obesity and fatty liver disease as signaling through this pathway has also been shown to improve glucose tolerance while reducing triglycerides and liver inflammation/fibrosis.<sup>58, 59, 63</sup> In a randomized, placebo-controlled, phase IIb trial, obeticholic acid, a synthetic variant of chenodeoxycholic acid and selective FXR agonist, was shown to significantly improve liver histology scores after 72 weeks of treatment in patients with non-alcoholic steatohepatitis.<sup>63</sup> However, at the end of the study LDL-C was significantly increased in the patients treated with obeticholic acid, demonstrating that the consequences of FXR activation in the regulation of lipid metabolism are confounded by the centrality of FXR in regulating liver physiology. The variability in production of primary:secondary bile acids by gut bacteria may influence the extent of FXR activation and subsequent FGF19 production.

The advanced technologies now available for profiling the microbiota composition provide a unique opportunity to examine the microbiome as a contributor to drug variability and characterize patients that would be considered non-responders, allowing for a precision medicine approach for the treatment of CVD. Phenotyping subjects based on their clinical and metagenomics response to statins may help identify the cause of suboptimal statin response and potential areas for intervention. Manipulation of the microbiota or gut-derived bile acids may also be a potential innovative therapeutic approach.

## 1.2 Name and description of the investigational product

Participants will be randomized to receive a placebo pill or rosuvastatin 20 mg per day. Rosuvastatin is FDA approved for the treatment of hypercholesterolemia. Participants of Asian ancestry will receive 10 mg daily.

### 1.2.1 Nonclinical data

Treatment of mice with rosuvastatin modulated the composition of the bacterial species, and reduced hepatic expression of the CYP27a1,<sup>9</sup> which encodes an enzyme regulating the conversion of 7 $\alpha$ -hydroxycholesterol to chenodeoxycholic acid, a primary bile acid.<sup>38-40</sup> Rosuvastatin, by inducing gene expression changes, altered the concentrations of bile acids which contribute to the composition of gut microbiota.

### 1.2.2 Clinical Data to Date

A proof-of-concept study in human subjects with hyperlipidemia showed that 4-8 weeks of rosuvastatin 10 mg significantly altered the gut microbiome and the abundance of specific bacterial taxa which was correlated to the LDL-C lowering response of the drug.<sup>12</sup> The phyla *Firmicutes* and *Fusobacteria* were negatively associated with LDL-C levels, but *Cyanobacteria* and *Lentisphaerae* were positively associated. However, this observational study lacked a control group, and the bacterial sequencing was performed only after treatment, thus the investigators were unable to assess the change in the microbiome.

## 1.3 Dose Rationale

Rosuvastatin 20 mg has been shown to reduce LDL-C by 47-57% at 6-8 weeks in patients with mild to moderate hypercholesterolemia.<sup>64, 65</sup> Rosuvastatin 20 mg is well tolerated with the following adverse event reported in  $\geq 2\%$  of clinical trial participants: nausea (6.3%), myalgia (6.3%), asthenia (4.7%), constipation (4.7%) and headache (3.1%).<sup>66</sup> In rare cases, myopathy and rhabdomyolysis with acute renal failure have been reported, with increased risk at the 40 mg dose.<sup>66</sup> In the Jupiter trial, where >17,000 healthy subjects were randomized to rosuvastatin 20 mg daily or placebo for 1 year, the rates of myalgia, arthralgia, nausea and constipation were similar between the groups.<sup>67</sup> Rosuvastatin 10 mg daily will be initiated in

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participants of Asian ancestry due to a 2-fold elevation in rosuvastatin exposure (AUC and C<sub>max</sub>) in Asian subjects.<sup>66</sup>

## 2. Study objectives

The objective of this study is to understand the interaction between the gut microbiome and host drug response to statin therapy using 16S rRNA sequencing, metagenomics sequencing and bile acid metabolomics. *The central hypothesis is that statins alter the gut microbiome composition and increase the bacterial enzyme-catalyzed deconjugation and/or dihydroxylation of primary bile acids, and thus increase signaling through the FXR-FGF19 axis, which contributes to the variability in change in LDL-C.*

Aim 1: To compare changes in the gut microbiome in healthy volunteers randomized to an 8-week intervention with rosuvastatin 10mg daily or placebo.

*Hypothesis 1A: Rosuvastatin significantly alters the gut microbiome composition towards increased abundance of bacteria with greater bacterial bile salt hydrolase activity (bsh).*

*Hypothesis 1B: Individual changes in plasma LDL-C levels with rosuvastatin is correlated with abundance of bacteria with increased bsh activity.*

Aim 2: To determine the relationship with gut microbiome, fecal bile acid composition, serum FGF19 levels and the change in plasma LDL-C with rosuvastatin.

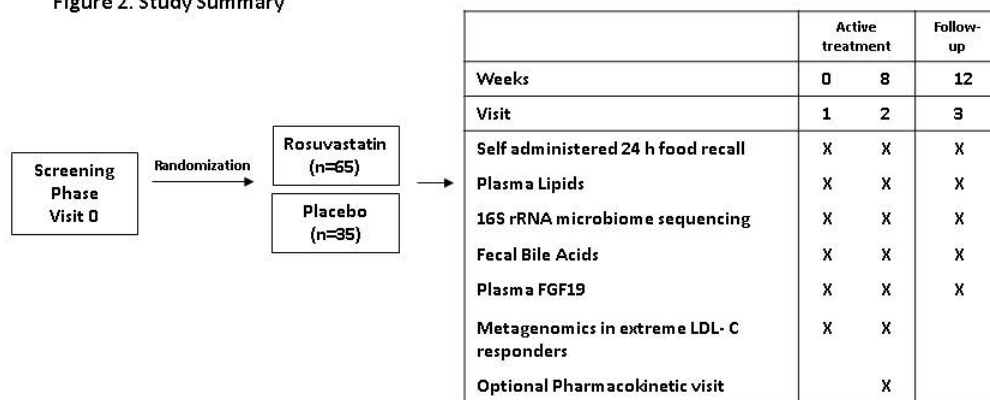
*Hypothesis 2: The change in plasma LDL-C levels with rosuvastatin is correlated with variation in circulating FGF19 levels, which reflects ileal exposure to bile acids.*

## 3. Study Design

### 3.1 General Design

This is a randomized, placebo controlled trial to investigate the effects of rosuvastatin on the gut microbiome, fecal bile acids and FGF19 levels. Healthy volunteers will be randomized to rosuvastatin 20 mg daily or placebo for eight weeks in a 2:1 ratio. Participants will be blinded to treatment assignment. There will be 3 study visits over the course of 16 weeks (Figure 2). Stool and blood will be collected at baseline, 8 weeks, and 12 weeks for 16S sequencing, plasma lipid assays, bile acid metabolites and FGF19 assays. A subgroup of participants at the tails of LDL-C response will undergo metagenomics sequencing.

Figure 2. Study Summary



### 3.1.1 Screening phase

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Subjects will be primarily recruited from the Preventive Cardiology Research databases, comprised of healthy subjects who have participated in previous research studies and who have asked to be contacted for future studies. The healthy volunteer database currently contains ~600 subjects. Healthy subjects have been successfully enrolled into pharmacological interventional studies from this database. Subjects may also be recruited via local IRB-approved flyers, radio, internet (eg. iConnect, Penn Medicine's Clinical Research Facebook page, etc.), UPHS databases, and newspaper advertisements targeting the Delaware Valley region. Ads will be submitted to the IRB for approval before use.

Potential participants will be screened first by a telephone interview by the clinical research coordinator (CRC) or an online survey administered via RedCap, completing a series of questions to determine eligibility. Potential subjects eligible based on these criteria will be brought in for an in-person screening visit (V0) at the Center for Human Phenomic Sciences (CHPS) where labs will be conducted as described in the inclusion/exclusion criteria section of the protocol. Written consent will be obtained before the screening labs are conducted.

### **3.1.2 Study Intervention Phase**

Eligible participants will return to CHPS for the baseline visit (V1) and randomization. As gut microbiota composition is associated with diet,<sup>68</sup> participants will complete 3 Automated Self-Administered 24-h recalls (ASA24) to document dietary intake on 3 days prior to V1.<sup>69</sup> The ASA24 was found to provide the best estimates for dietary intake and outperformed food frequency questionnaires. They will be instructed to collect a stool sample at home 24 hours prior to all study visits. Blood will be drawn for plasma lipid assessment and FGF19 levels at V1-V3. A urine sample will be collected at V1, V2, and V3 and stored for metabolomic studies. They will receive an 8 week supply of study drug or matched placebo at V1. Study staff will contact the subjects between V1 and V2 (after 4 weeks of treatment) to discuss any AEs or compliance issues. Drug compliance will be assessed at V2. Drug-related adverse events will be assessed at V2 and V3. Participants will again complete the ASA24 on 3 separate days during the week of V2.

### **3.1.3 Follow up Phase**

Participants will be brought in for 1 visit (V3) after discontinuation of the study drug to evaluate drug-related adverse events and final sample collection. Participants will complete the ASA24 on 1 additional day during the week of V3. This final stool sample will provide information about recovery of the gut microbiome after the drug has been stopped. Total involvement will be about 14-16 weeks including the screening period.

### **3.1.4 Allocation to the Interventional Group**

Participants will be randomized to the study intervention. The biostatistician will employ the random number generator within Stata V13.1 (College Lake, TX) to create group assignments which will be placed in sequentially numbered sealed envelopes. The envelope seal will be broken by the research coordinator after participant consent, revealing group allocation. Randomization will be stratified by sex and race.

### **3.1.5 Rosuvastatin Pharmacokinetic (PK) Substudy**

Participants consenting to the optional PK substudy will be admitted to the CHPS outpatient unit for a 12 hour visit on V2 in place of their regular study visit. We expect that up to 40 participants will complete the substudy. Participants will be instructed to fast for at least 8 hours before the visit and hold their usual dose of study drug. They will receive the study drug in the CHPS unit at a target time of 0700 and 12 venous blood samples will be collected over a 12 hour period.

### 3.2 Study Endpoints

#### 3.2.1 Primary Study Endpoints

- The change from baseline to 4 weeks, from **baseline to 8 weeks (primary)** and 8 weeks to 12 weeks (recovery) in bacterial abundance as measured by Operational Taxonomic Units (OTUs).<sup>70</sup>
- The change in bacteria composition or beta diversity as measured by weighted UniFrac distance.<sup>71, 72</sup>

#### 3.2.2 Secondary Study Endpoints

- The change in plasma LDL-C levels
- The change in fecal bile acid concentrations
- The change in serum FGF19 levels

#### 3.2.3 Primary Safety Endpoints

Not applicable

#### 3.2.4 Pharmacokinetic Study Endpoints

- Rosuvastatin systemic exposure as measured as area under the curve (AUC) from 0 to 10 hours (AUC<sub>0-10</sub>) and 0 to infinity (AUC<sub>0-∞</sub>).
- Rosuvastatin peak plasma concentration (C<sub>max</sub>) and time to peak concentration (T<sub>max</sub>), mean absorption time (MAT).

## 4. Study Population and Duration of Participation

### 4.1 Inclusion Criteria

1. Participant is capable of giving informed consent
2. Participant is aged 18 to 65 years. The gut microbiome has been shown to change gradually with time, although there is no cut-off in age when this occurs.<sup>73</sup>

### 4.2 Exclusion Criteria

1. Participants with cardiovascular disease such as a history of heart failure (New York Heart Association class II-IV), myocardial infarction, stroke, coronary artery bypass graft, hypertension, and hyperlipidemia as these conditions are associated with altered gut microbiome composition.<sup>74</sup> Hypertension is defined as blood pressure greater than 160/110 or on any anti-hypertensive medications. LDL-C >190 mg/dl and triglycerides > 400 mg/dl.
2. Participants with a history of cancer.
3. Kidney disease (serum creatinine >1.5 mg/dl).
4. Liver dysfunction (alanine aminotransferase > 2 times the upper limit of normal).
5. Diabetes mellitus (DM) – Diabetes itself may affect the gut microbiome although this has not been extensively studied. In addition to a prior diagnosis of diabetes mellitus other than that related to pregnancy, a fasting glucose level of greater than 125mg/dL will be used to exclude participation.
6. Clinical diagnosis of hypothyroidism
7. History of inflammatory disorders of the intestinal tract (i.e. IBD, celiac sprue).
8. Use of antibiotics in the prior 3 months.
9. Use of pre-, pro-, or synbiotics.

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10. Chronic medication use for the treatment of cardiovascular disease, cancer, kidney disease, liver disease, gastrointestinal disease, diabetes and thyroid disease. Since we are evaluating the impact of rosuvastatin on the gut microbiome we would like to exclude the potential impact of confounding medications. Certain classes of medication are allowable including oral contraceptives, antidepressants and allergy medications. Each medication will be evaluated by the study PI for its potential impact on the gut microbiome.
11. Current smoker. The effect of smoking on the microbiome of the gut is unknown.
12. Known history of alcohol or substance abuse.
13. Body Mass Index (BMI) <18.5 or > 35 kg/m<sup>2</sup>. Volunteers with BMI below normal will be excluded to prevent inclusion of subjects with a subclinical systemic disease that may influence the gut microbiome. Volunteers with moderate or severe obesity will be excluded as obesity may be associated with altered gut microbiome composition.<sup>31</sup>
14. Unable to abstain from consumption of illicit drugs during the study period.
15. Prior bowel resection surgery other than appendectomy. It is unknown how prior bowel resection surgery may influence the microbiome composition; hence we will exclude these participants.
16. Baseline bowel frequency less than every 2 days or greater than 3 times daily. Normal bowel frequency is every 3rd day to 3 times per day. Although unknown, stool frequency could be related to the microbiome composition. To avoid the need for use of antidiarrheal medications or laxatives, which themselves could alter the microbiome composition, these patients will be excluded.
17. Participant has experienced diarrhea within the two weeks prior to entry. Diarrhea is defined as a change in bowel habits with an increased frequency or loose stools such that the stool could not be lifted with a fork.
18. Vegans and Vegetarians.
19. Known intolerance to statin medications.
20. Unwilling to obtain from grapefruit containing foods or drinks.
21. Pregnant women. To avoid any risk to an unborn fetus from study drug exposure.
22. Refusal to use two medically accepted method of birth control while participating in the study, such as a barrier method, hormonal contraceptives, implanted birth control devices, permanent methods (such as a vasectomy), and/or abstinence.
23. Nursing mothers
24. Any condition that the investigator feels may limit the volunteer's ability to complete the study protocol.

#### 4.3 Participant recruitment and screening

Subjects will be primarily recruited from the Preventive Cardiology Research databases, comprised of healthy subjects who have participated in previous research studies and who have asked to be contacted for future studies. The healthy volunteer database currently contains ~600 subjects. Healthy subjects have been successfully enrolled into pharmacological interventional studies from this database. If needed, additional subjects will also be recruited via local IRB-approved flyers, radio, internet and newspaper advertisements.

Potential participants will be screened for eligibility before being enrolled in the study. Potential participants will be directly queried regarding exclusion criteria. If there are questions regarding eligibility, relevant information from the participant's medical record will be sought from their treating physician. In the absence of any concerns, the participant's medical records will not be requested or reviewed as part of this study.

#### 4.4 Duration of study participation

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Total involvement will be about 14-16 weeks, depending on the length of the screening period.

#### **4.5 Total number of subjects**

This is a single center study. We will enroll 100 subjects with the goal of 90 completers; accounting for a 10% drop-out rate.

## **5. Study intervention**

### **5.1 Description**

Rosuvastatin 20 mg is approved by the US FDA and available by prescription only. The drug can be taken with or without food. Rosuvastatin 10 mg will be used in participants of Asian ancestry.

### **5.2 Intervention regimen**

Participants will take rosuvastatin or placebo pill by mouth once daily in the morning for 8 weeks.

### **5.3 Preparation and packaging**

The Investigation Drug Service (IDS) will package blinded study medications according to the Hospital of the University of Pennsylvania's Pharmacy Policy and Procedures. Labeling will be done by the IDS, and each packet will include the patient's unique identification number, name, date dispensed, storage conditions and directions for use. The study staff will document all oral doses dispensed in the subject's chart.

### **5.4 Blinding**

Participants will be blinded to the treatment assignment (single-blind).

### **5.5 Administration and accountability**

The IDS service will maintain accurate logs of study drug dispensing, and will conduct regular drug reconciliation checks to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the service.

### **5.6 Subject compliance monitoring**

At each study visit, compliance with study drug will be reinforced. When study drug is returned, compliance will be assessed by pill count. Study medication dispensed or returned to IDS must be recorded for Drug Accountability and Reconciliation at each visit.

#### **5.6.1 Return or destruction of investigational product**

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

## **6. Study procedures**

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The study procedures section is divided into two parts, one describing the collection methods of samples for metabolic and microbial profiling and the second describing the sequence of events.

## 6.1 Sample collection methods for metabolic and microbial profiling

### 6.1.1 Blood sample collection and storage

Up to 30 cc of blood will be collected for blood chemistries, complete blood count, plasma lipid measurements and FGF19 assays. Plasma, serum, and DNA will be separated from the blood samples and stored frozen at -80°C for use in future metabolic and genetic studies.

### 6.1.2 Stool sample collection

Participants will be instructed on home stool collection methods and will be required to collect a stool sample and bring it with them to each of the V1-V3 study visits. Study staff will provide the participant with collection materials, packaging and instructions for collecting the stool at home and bringing it to the study visits (Appendix A). Participants will be provided with ice packs and a container in which to store the sample. Immediately after voiding the sample, the participant will rate the stool on the Bristol Stool Chart (Appendix B). The study coordinator will bring this stool sample to the study lab on the 11th floor Smilow Center for Translational Research (SCTR). Lab personnel will aliquot the stool sample into a minimum of 6 vials. One sample will be used for microbiome sequencing and the remaining samples will be frozen at -80°C for fecal bile acid metabolomics.

### 6.1.3 Urine sample collection and storage

There is increasing evidence that the composition of the gut microbiome influences the metabolic phenotype of blood, urine, and other tissues.<sup>75</sup> Urine samples will be collected at V1, V2 and V3 for future metabolomics studies.

## 6.2 Sequence of events

**Table 1. Sequence of study activities**

| Phase                          | Screening | Baseline visit | Phone call | Active Treatment | Follow-up |
|--------------------------------|-----------|----------------|------------|------------------|-----------|
| Week                           | -4 to -2  | 0              | 4          | 8                | 12        |
| Visit                          | 0         | 1              |            | 2                | 3         |
| Consent                        | x         |                |            |                  |           |
| Screening history              | x         |                |            |                  |           |
| Physical exam                  | x         |                |            |                  |           |
| CBC                            | x         |                |            |                  |           |
| CMP (includes liver function)  | x         |                |            | x                |           |
| Pregnancy test                 | x         |                |            |                  |           |
| Lipid panel                    | x         | x              |            | x                | x         |
| Randomization                  |           | x              |            |                  |           |
| Dispensing of study drug       |           | x              |            |                  |           |
| Self-assessed 24-h food recall |           | x3             |            | x3               | x1        |
| Serum for FGF19                |           | x              |            | x                | x         |
| Stool sample                   |           | x              |            | x                | x         |
| Urine sample                   |           | x              |            | x                | x         |
| DNA sample                     |           | x              |            |                  |           |
| Optional Pharmacokinetic Visit |           |                |            | x                |           |

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### **6.2.1 Screening (-4 to -2 weeks, V0)**

Screening evaluation should be completed no more than 30 days prior to baseline visit/randomization. After providing informed consent, participants will undergo a medical history to assess for exclusion criteria. Demographic data collected will include age, sex, race, ethnicity, lifetime weight history, current and recent medication use, past medical and surgical history, tobacco use, and alcohol use.

#### **6.2.2.1 Physical exam**

The physical exam will document any baseline abnormalities as well as the baseline BMI, and a seated blood pressure measurement.

#### **6.2.2.2 Laboratory studies**

Blood will be drawn for complete blood count (CBC), comprehensive metabolic panel which includes liver function tests, and the lipid panel. Women will complete a urine pregnancy test. All eligibility criteria will be reviewed.

### **6.2.3 Baseline visit (Week 0, V1)**

After reviewing laboratory results, eligible participants will be contacted to schedule a baseline visit. They will be instructed on how to collect a stool sample at home 24 hours prior to the visit. They will be instructed not to eat for at least 8 hours to ensure a fasting morning blood sample collection.

All participants will arrive at the CHPS in the morning, having not eaten for at least 8 hours. The stool sample collected at home will be given to the coordinator and blood samples will be obtained and processed as described above.

#### **6.2.3.1 Automated Self-Administered 24-h (ASA24) Dietary Assessment Tool.**

We will collect information on the participant's usual diet utilizing the Automated Self-Administered 24-Hour (ASA24®) Dietary Assessment Tool, developed by the National Cancer Institute (NCI).<sup>76</sup> The ASA24 is a web-based dietary assessment instrument that asks participants to recall and record all foods and drinks they consume in a 24-hour period. It has been found to be more accurate and minimizes underreporting compared with food frequency questionnaires.<sup>69</sup> Participants can also use the ASA24 on their mobile devices to enter food and drinks as they are consumed to minimize underreporting. They will complete 3 days' worth of intake during the week of V1 and V2 and 1 days' worth during the week of V3 to estimate average intake during the course of the study.

#### **6.2.3.2 Randomization**

The research coordinator will allocate the participants to the treatment group as described above.

#### **6.2.3.3 Administration of study drug**

The research coordinator will provide an 8 week supply of the study drug and instruct participants on how to take the medication. The participants will be provided with an adverse event/daily pill log to record any adverse events related to the study medication, counseled on signs and symptoms of drug related adverse events and who and when to call to seek medical attention for adverse events. The participants will be contacted by phone after 4 weeks of treatment to discuss any AEs or compliance issues.

#### **6.2.4 Study visit [Week 8 (V2)]**

The research coordinator will remind participants to collect a stool sample at home 24 hours prior to the visit. They will be instructed not to eat for at least 8 hours to ensure a fasting morning blood sample collection.

All participants will arrive at the CHPS in the morning, having not eaten for at least 8 hours. The stool sample collected at home will be given to the coordinator and blood samples will be obtained and processed as described above.

The research coordinator will review the adverse event/daily pill log and study medication compliance.

#### **6.2.5 Optional Pharmacokinetic Visit [Week 8 (V2)]**

The research coordinator will remind participants to collect a stool sample at home 24 hours prior to the visit. They will be instructed not to eat for at least 8 hours to ensure a fasting morning sample and hold their morning dose of study drug.

Participants will arrive at the CHPS in the morning. The stool sample collected at home will be given to the coordinator. They will receive the study drug in the CHPS unit at a target time of 0700 and venous blood samples (3 mL each) will be collected at t=0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hours post dosing. Blood will be centrifuged at 4 °C and aliquoted plasma samples will be stored at -80 °C until bioanalysis.

#### **6.2.6 Follow-up visit [Week 12 (V3)]**

The research coordinator will remind participants to collect a stool sample at home 24 hours prior to the visit. They will be instructed not to eat for at least 8 hours to ensure a fasting morning blood sample collection.

All participants will arrive at the CHPS in the morning, having not eaten for at least 8 hours. The stool sample collected at home will be given to the coordinator and blood samples will be obtained and processed as described above.

The research coordinator will review adverse events any safety concerns since withdrawal of the study medication.

### **6.3 Early withdrawal of Participants**

Participants may be withdrawn from the study at any time if the investigator or the participant believes that the participant's safety will be jeopardized by continued participation, or if the participant does not follow study procedures. Likewise, participants will be withdrawn from the study if they withdraw consent.

## **6.4 Sample preparation, processing and storage**

### **6.4.1 Aliquoting and processing of samples for archiving**

Stool samples are aliquoted into spoon-capped vials labeled with a barcode that is a unique identifier, the date and a protocol number, usually 3 per sample, for archiving. Any residual stool is aliquoted for specific immediate demands by investigators and/or removed from the collection vessel to 3 x 15 mL vials, which is also labeled with a similar barcode. Each sample is then scanned, and placed in an -80°C freezer in an appropriate freezer box with its own separate barcode. The freezer box barcode is scanned at the same time. Blood samples are processed for either serum or plasma, depending on the preservatives added at the time of sampling. Once in the lab, the blood is centrifuged to clear the plasma/serum and the supernatant is measured out in 300-500 µL aliquots into polypropylene cryotubes. Each aliquot is labeled in the same manner as the stool aliquots and immediately placed in a barcoded box in

an -80°C freezer to be archived for later use. Finally, when retrieving a stored sample the box and aliquot barcodes are scanned and compared to a list of samples needed to ensure the correct material is being removed for use.

## 6.4.2 Microbiome characterization

### 6.4.2.1 16s rRNA sequencing

Processing and Bacterial DNA sequencing will be performed by research staff within the Microbiome Core at UPenn (BRB 9<sup>th</sup> Floor). DNA extraction from fecal samples will be performed using the Qiagen DNeasy Power Soil Kit. Isolated DNA will be quantified using the PicoGreen system. Remaining DNA will be stored at -80°C for future studies. Bacterial 16S rRNA sequencing will be processed using Quantitative Insights Into Microbial Ecology (QIIME), which was co-written by members of the PennCHOP Microbiome Core.<sup>77</sup> Primers annealing to the V1-V2 region of the 16S rRNA gene will be used for amplification as described previously.<sup>78</sup> DNA-free water will undergo the same amplification and purification procedure to allow for empirical assessment of environmental and reagent contamination. Positive controls, consisting of eight artificial 16S gene fragments synthesized in gene blocks and combined in known abundances, will also be included. Initial bioinformatics processing will be completed by bioinformaticians in microbiome core. Operational Taxonomic Units (OTUs), which are surrogates for bacterial taxa defined by 16S rRNA homology, will be selected by clustering reads at 97% sequence similarity.<sup>79</sup> Taxonomic assignments will be generated by comparison to the Greengenes reference database<sup>80</sup>, using the consensus methods in QIIME. A phylogenetic tree will be inferred from the OTU data using FastTree.<sup>81</sup>

### 6.4.2.2 Metagenomics sequencing

Metagenomics sequencing will be performed in a subset of participants at the extremes of LDL-C response. Metagenomics processing and analyses will be performed by the technicians and bioinformaticians in the Microbiome Core. Shotgun libraries will be generated from 1 ng of DNA using the NexteraXT kit (Illumina, San Diego, CA, USA). Libraries will be sequenced on the Illumina HiSeq using 2x125 bp chemistry in High Output mode. Extraction blanks and DNA free water will be included to empirically assess environmental and reagent contamination. Laboratory-generated mock communities consisting of DNA from *Vibrio campbellii*, *Cryptococcus diffluens*, and Lambda phage will be included as positive sequencing controls. Paired-end sequence data will be quality filtered to remove adapter sequence and low quality reads. Human-derived reads will be removed by alignment to human reference genomes and a custom database of human-associated sequences. The abundance of bacterial taxa will be estimated with MetaPhlAn2.<sup>82</sup> The abundance of genes present will be assessed by alignment to the KEGG reference database.<sup>83</sup> Reads will be aligned to a set of reference genomes from NCBI to determine genome coverage and assess differences between observed and reference genomes.

### 6.4.3 Bile acid metabolomic analyses of fecal samples

Targeted Metabolite measurement by Liquid-Chromatograph/Mass Spectrometry will be performed by the PennCHOP Metabolomics Core (Director, Dr. Gary Wu). The core has validated methods to assay the metabolites listed in Table 2 as previously described.<sup>84</sup> Bile acids will be quantified using a Waters

| Table 2. List of Targeted Metabolites to be Assayed |                         |               |
|-----------------------------------------------------|-------------------------|---------------|
| Bile acids                                          | Short chain fatty acids | Amino acids   |
| Cholic acid                                         | Acetic acid             | Alanine       |
| Glycocholic acid                                    | Butyric acid            | Arginine      |
| Taurocholic acid                                    | Formic acid             | Aspartic acid |
| Chenodeoxycholic acid                               | Hepanoic acid           | Cysteine      |
| Glycochenodeoxycholic acid                          | Hexanoic acid           | Glutamine     |
| Taurochenodeoxycholic acid                          | Isobutyric acid         | Histidine     |
| Deoxycholic acid                                    | Isovaleric acid         | Isoleucine    |
| Glycodeoxycholic acid                               | 4-Methylvaleric acid    | Leucine       |
| Lithocholic acid                                    | Propionic acid          | Lysine        |
| Glycolithocholic acid                               | Valeric acid            | Methionine    |
| Tauroolithocholic acid                              |                         | Phenylalanine |
|                                                     |                         | Proline       |
|                                                     |                         | Serine        |
|                                                     |                         | Threonine     |
|                                                     |                         | Tryptophan    |
|                                                     |                         | Tyrosine      |
|                                                     |                         | Valine        |

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Acquity uPLC System with a QDa single quadrupole mass detector and an autosampler (192 sample capacity). Briefly, fecal samples are suspended in methanol (5  $\mu$ L/mg stool), vortexed, centrifuged and supernatant collected. Samples are analyzed on an Acquity uPLC with a Cortecs UPLC C-18+ 1.6 mm 2.1 x 50 mm column with flow rate of 0.8 mL/min, injection volume 4  $\mu$ L, column temperature 30°C, sample temperature 4°C, and run time 4 min per sample. Samples are eluted with 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Samples are quantified against standard curves of at least five points run in triplicate. Standard curves are run at the beginning and end of each metabolomics run. Quality control checks (blanks and standards) are run every eight samples. Results are rejected if the standards deviate by greater than  $\pm 5\%$ . The range of the assay is at least 50 nM – 10,000 nM; the limit of detection is < 50 nM, and; the limit of quantitation is >10,000 nM. Bile acid concentrations will be normalized and log transformed prior to analysis.

#### 6.4.4 Quantification of fibroblast growth factor 19 (FGF19)

Serum FGF19 levels will be measured using sandwich ELISA kit (R&D systems, Minneapolis, MN) by research staff as specified by the manufacturer's instructions.

#### 6.4.5 Pharmacokinetic bioanalysis

Plasma samples will be analyzed for rosuvastatin, N-desmethyl rosuvastatin and rosuvastatin lactone by high performance liquid chromatography at PPD Laboratories (Richmond, VA).

#### 6.4.6 Long term sample storage and reuse of samples

All specimens remaining after the study is complete will be retained for possible future use unless specifically requested by the participant that the specimen be destroyed or it is deemed by the investigators that the specimens are no longer needed.

## 7 Statistical Plan

### 7.1 Sample size

We will enroll 100 healthy subjects, randomized in a 2:1 fashion to rosuvastatin or placebo, with the goal of 90 completers; accounting for a 10% drop-out rate. We will stratify enrollment based on sex and race. For the primary aim, we will examine the effect of treatment on the change in bacterial taxa. Based on a paired t-test, enrolling 60 subjects in the rosuvastatin arm, will provide power of 0.80 to detect a Cohen's d effect size of 0.8, assuming 40 relatively common species will be tested at an alpha level of 0.001 (or 0.05/40 for multiple comparison adjustment using Bonferroni) (Figure 3, red line). For aim 2, based on a paired t-test, enrolling 60 subjects in the rosuvastatin arm in Aim 1, will provide power of 0.80 to detect a Cohen's d effect size of 0.8, measuring 12 bile acid metabolites at an alpha level of 0.004 (or 0.05/12 for multiple comparison adjustment using Bonferroni) (Figure 3, green line).

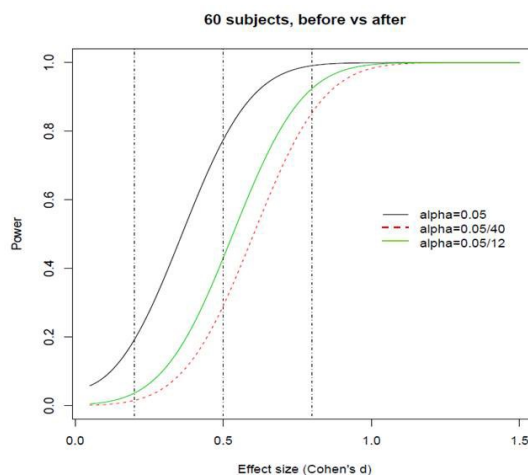


**Figure 3. Power Curve.** The power estimates for enrollment of 60 subjects in the treatment group, with multiple testing correction for 40 taxa in Aim 1 (red line) and 12 bile acids in Aim 2 (green line). Vertical dashed lines represent weak, medium and large effect sizes.

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## 7.2 Overview of statistical methods

Descriptive statistics (mean, SD, median, range, counts, and percentage) will be used to describe and compare (t-test or rank sum test for continuous variables and Fishers exact test for categorical variables) the baseline characteristics, usual diet between the treatment arms.

### 7.2.1 Determining the effect of study medication on fecal microbiome composition

From the microbiome sequencing, we will obtain tables of OTUs which will be used to determine the relative abundance of taxa in each group. In addition, we will compare beta diversity, the degree of difference between bacterial communities measured as a continuous distance, in statin versus placebo treated and the change pre- and post-treatment. Weighted UniFrac distance (distance measurement which incorporates phylogenetic relationship, weighted by abundance),<sup>71, 72</sup> is the primary outcome we will use to examine beta diversity. The primary analysis will compare the change in beta diversity between statin-treated to placebo from baseline to eight weeks. In secondary analyses, we will examine the change from baseline to four weeks. We will use weighted UniFrac distance for the primary outcome visualized with Principal Coordinates Analysis (PCoA) to analyze the abundance of genes and taxa at the community level. The overall difference of the microbiome composition will be testing using permutation distance-based multivariate analysis of variance (PERMANOVA).<sup>85</sup> Non parametric rank tests will be used to detect differences in taxon abundance between sample groups. Differential abundance of specific taxa will be assessed using nonparametric rank tests or generalized linear mixed effects models. To adjust for potential confounders and to evaluate the association between clinical data (e.g. age, sex, body mass index, diet) and microbial community profiles, we will use the microbiome regression-based kernel association test (MiRKAT).<sup>86</sup> The association between changes in microbial composition with clinical characteristics (sex, race, age, BMI, diet) and outcome (the change in LDL-C) will be assessed using multivariate modeling. The generalized linear models will be selected when within vs. between-subject effects will be characterized. False discovery rate (FDR) of 5% will be applied to account for multiple comparisons.

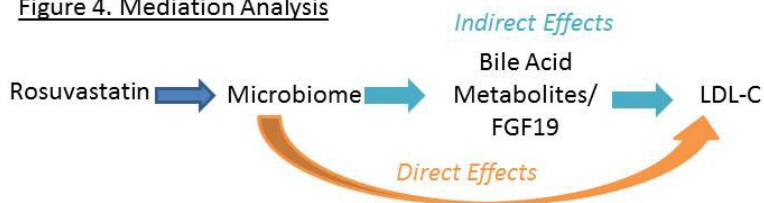
### 7.2.2 Functional Profiling from metagenomics sequencing data

First we will apply a computational approach called PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) to predict the functional composition of a metagenome using marker gene data from 16S data and a reference genome database.<sup>87</sup> Our findings will be validated by functional profiling of the metagenomics profiles acquired from the extreme responders to rosuvastatin. HUMAnN2<sup>88</sup> will be used to calculate gene and pathway abundance from metagenomic sequencing data. MetaCyc will be used for pathway analysis.<sup>89</sup> Quantile normalization will be performed and genes/pathways with low present rate (<10% of samples) will be removed.

### 7.2.3 Determining the effect of study medication on fecal bile acids and FGF19 concentrations

The changes in bile acid metabolites will be examined individually, grouped as primary or secondary bile acid or as the ratio of primary:secondary bile acids over time in response to treatment. Mixed-effects models will be established to identify metabolites that change,

Figure 4. Mediation Analysis



adjusting for covariates with FDR controlling for multiple comparisons.<sup>90</sup> A similar analysis will be performed to evaluate changes in FGF19 over time. General linear mixed effects models will be constructed with delta LDL as the dependent variable,

including bile acids levels in the model and adjusting for BMI, gender, and diet. Similarly, models will be constructed to evaluate the contribution of FGF19 to delta LDL levels. For taxa and metabolites we identify, we will also apply within subject mediation analysis (Figure 4) to explore the underlying mechanism (e.g. changes in bile acid metabolite levels, FGF19) by which the microbiome influences LDL-C. Change in taxa abundance will be used as the exposure, change in bile acids or FGF19 as the mediators and delta LDL-C as the outcome. We will quantify the direct effect and indirect mediating effects of taxa on delta LDL-C and explain the process that underlies the relationship between the microbiome, bile acid metabolites, FGF19 and LDL-C.

### 7.2.4 Pharmacokinetic analysis

Rosuvastatin pharmacokinetic parameters will be estimated from plasma concentration data by noncompartmental analysis. The  $AUC_{0-\infty}$  will be calculated using the trapezoidal method. Rosuvastatin  $T_{max}$  and  $C_{max}$  will be directly obtained from observed data. Oral clearance (CL/F) will be calculated as  $dose / AUC_{0-\infty}$ . We will determine if bacterial species, bile acid metabolites are predictive of rosuvastatin AUC.

## 8 Safety and adverse events

### Adverse Event

An **adverse event** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

### Serious Adverse Event (SAE)

Adverse events are classified as serious or non-serious. A **serious adverse event** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay

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- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the participant, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious. All adverse events that do not meet any of the criteria for serious will be regarded as **non-serious adverse events**.

#### ***Adverse Event Reporting Period***

The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 4 weeks following the last administration of study medication.

#### ***Preexisting Condition***

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

#### ***General Physical Examination Findings***

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an AE must also be recorded and documented as an AE.

#### ***Post-study Adverse Event***

All unresolved AEs should be followed by the investigator until the events are resolved, the participant is lost to follow-up, or the AE is otherwise explained. At the last evaluation, the investigator will instruct each participant to report any subsequent event(s) that the participant, or the participant's personal physician, believes might reasonably be related to participation in this study. The investigator will notify the IRB of any death or AE occurring at any time after a participant has discontinued or terminated study participation that may reasonably be related to this study. The IRB will also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a participant that has participated in this study.

#### ***Abnormal Laboratory Values***

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

#### ***Hospitalization, Prolonged Hospitalization or Surgery***

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Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

### **8.1 Recording of Adverse Events**

At each contact with the participant, the investigator must seek information on AEs by specific questioning and, as appropriate, by examination. Information on all AEs should be recorded immediately in the source document, and also in the appropriate adverse event case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All AEs occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study medication or study participation should be recorded and reported immediately.

### **8.2 Reporting of serious adverse events**

#### **8.2.1 Study investigator notification**

An SAE must be reported to the study investigators by telephone within 24 hours of the event. An FDA Medwatch 3500A SAE Form must be completed by the investigator. The investigator will keep a copy of this form on file at the study site. Report SAEs by phone to:

Sony Tuteja, PharmD  
Phone- (215) 573-7834  
Mobile- (484) 431-1002

In the event that Dr. Tuteja cannot be reached, report SAEs to:

Archna Bajaj, MD  
Phone- (215) 662-7197  
Mobile- (518) 421-1996

At the time of the initial report, the following information should be provided:

- |                              |                                                                                    |
|------------------------------|------------------------------------------------------------------------------------|
| • Study Name                 | • Whether the study treatment was discontinued                                     |
| • Participant number         | • The reason the event is classified as serious                                    |
| • A description of the event | • Investigator assessment of the association between the event and study treatment |
| • Date of onset              |                                                                                    |
| • Current status             |                                                                                    |

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Within the following 48 hours, the investigator must provide further information on the SAE in the form of a written narrative. This should include a copy of the completed Medwatch 3500 A SAE Form and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing SAEs should be provided promptly to the study investigator.

### **8.2.2 Ethics committee/ Investigational Review Board Notification by Investigator**

Reports of all SAEs (including follow-up information) must be submitted to the Ethics Committee (EC)/Investigational Review Board (IRB) within 10 working days. Copies of each report and documentation of EC /IRB notification and receipt will be kept in the Clinical Investigator's binder.

## **9 Data Handling and Record Keeping**

### **9.1 Confidentiality**

Information about study participants will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed participant authorization informing the participant of the following:

- What protected health information (PHI) will be collected from participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research participant to revoke their authorization for use of their PHI.

In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization.

All data related to this trial will be recorded using the patients' assigned unique study number. Data will be reported only in a confidential manner such that the personal identity of any subject will not be identifiable. All study data will be maintained under a double locked system, such as a locked closet within a locked office or on a password protected computer in a locked office. At the end of the study these data will be electronically archived on a password protected computer or other electronic storage device.

### **9.2 Source Documents**

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

### 9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

### 9.4 Records retention

Study documents and records will be retained for at least 2 years after the last participant has completed the study.

## 10 Study monitoring, auditing, and inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

## 11 Ethical considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent EC/ IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

### 11.1 Risks

Expected and unexpected study drug-related adverse events may be experienced by study participants. The following mild adverse events have been reported with **rosuvastatin** and will be documented in the participants' record including: headache, mild muscle aches or pains, constipation, nausea, and diarrhea. The study drug will be stopped if the participants experience a serious adverse event associated with **rosuvastatin** including: fever, unusual tiredness, dark colored urine; severe muscle pain, tenderness or weakness; confusion, memory problems. Blood draws may be associated with bleeding, infection and bruising at the access site. These risks are minimized by using trained personnel. Additionally, there is the risk of loss of privacy.

## **11.2 Benefits**

There is no direct benefit to subjects from involvement in these studies. Clinical data relating to general health will be provided to them and their primary physician if they so wish. The overall risk of the proposed research is low and therefore the overall risk to benefit ratio is acceptable.

## **11.3 Informed consent process**

All participants for this study will be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. See Attachment A for a copy of the Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a participant, using the EC/IRB-approved consent form, must be obtained before that participant is submitted to any study procedure. This consent form must be signed by the participant or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent. Potential subjects and the investigator or a member of her designated research staff will review and sign the informed consent form during screening visit (visit 0) in an appropriate room at the CHPS. As informed consent is an ongoing process, any new information that affects a person's willingness to continue with the trial or risk profile that is received after the trial has been initiated will be provided to all subjects, whether participation has been completed or is ongoing. In order to avoid undue influence or coercion, all subjects will be treated equally.

## **12 Study Finances**

### **12.1 Funding source**

This study is financed through a grant from the National Heart Lung and Blood Institute (NHLBI) of the National Institutes of Health (NIH).

### **12.2 Conflict of Interest**

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of Pennsylvania investigators will follow the University conflict of interest policy.

### **12.3 Participant Payments**

Participants will be compensated \$225 for completion of the study. Specifically, study visits will be prorated as follows: V1- \$75, V2-\$75, V3-\$75. If needed, participants will also receive a \$10 parking voucher for study visits. Participants will be compensated an additional \$100 if they choose to participate in the optional pharmacokinetic visit.

## **13 Publication and Data Sharing Plan**

Data generated by this study will be shared at scientific meetings and published in scientific journals. Reasonable request for samples and data will be shared in a de-identified manner.

## 14 References

1. Koren O, Spor A, Felin J, Fåk F, Stombaugh J, Tremaroli V, Behre CJ, Knight R, Fagerberg B, Ley RE, Bäckhed F. Human oral, gut, and plaque microbiota in patients with atherosclerosis. *Proceedings of the National Academy of Sciences*. 2011;108:4592-4598
2. Karlsson FH, Fak F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, Backhed F, Nielsen J. Symptomatic atherosclerosis is associated with an altered gut metagenome. *Nature communications*. 2012;3:1245
3. Caesar R, Nygren H, Oresic M, Backhed F. Interaction between dietary lipids and gut microbiota regulates hepatic cholesterol metabolism. *J Lipid Res*. 2016;57:474-481
4. Fu J, Bonder MJ, Cenit MC, Tigchelaar EF, Maatman A, Dekens JA, Brandsma E, Marczyńska J, Imhann F, Weersma RK, Franke L, Poon TW, Xavier RJ, Gevers D, Hofker MH, Wijmenga C, Zhernakova A. The gut microbiome contributes to a substantial proportion of the variation in blood lipids. *Circ Res*. 2015;117:817-824
5. Haiser HJ, Gootenberg DB, Chatman K, Sirasani G, Balskus EP, Turnbaugh PJ. Predicting and manipulating cardiac drug inactivation by the human gut bacterium *eggerthella lenta*. *Science*. 2013;341:295-298
6. Clayton TA, Baker D, Lindon JC, Everett JR, Nicholson JK. Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. *Proc Natl Acad Sci U S A*. 2009;106:14728-14733
7. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Manneras-Holm L, Stahlman M, Olsson LM, Serino M, Planas-Felix M, Xifra G, Mercader JM, Torrents D, Burcelin R, Ricart W, Perkins R, Fernandez-Real JM, Backhed F. Metformin alters the gut microbiome of individuals with treatment-naïve type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med*. 2017;23:850-858
8. Spanogiannopoulos P, Bess EN, Carmody RN, Turnbaugh PJ. The microbial pharmacists within us: A metagenomic view of xenobiotic metabolism. *Nature reviews. Microbiology*. 2016;14:273-287
9. Nolan JA, Skuse P, Govindarajan K, Patterson E, Konstantinidou N, Casey PG, MacSharry J, Shanahan F, Stanton C, Hill C, Cotter PD, Joyce SA, Gahan CGM. The influence of rosuvastatin on the gastrointestinal microbiota and host gene expression profiles. *Am J Physiol Gastrointest Liver Physiol*. 2017;312:G488-G497
10. Khan TJ, Ahmed YM, Zamzami MA, Mohamed SA, Khan I, Baothman OAS, Mehanna MG, Yasir M. Effect of atorvastatin on the gut microbiota of high fat diet-induced hypercholesterolemic rats. *Scientific reports*. 2018;8:662
11. Fu ZD, Cui JY, Klaassen CD. Atorvastatin induces bile acid-synthetic enzyme *cyp7a1* by suppressing *fxr* signaling in both liver and intestine in mice. *J Lipid Res*. 2014;55:2576-2586
12. Liu Y, Song X, Zhou H, Zhou X, Xia Y, Dong X, Zhong W, Tang S, Wang L, Wen S, Xiao J, Tang L. Gut microbiome associates with lipid-lowering effect of rosuvastatin in vivo. *Frontiers in microbiology*. 2018;9:530
13. WRITING GROUP MEMBERS, Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosamond W, Sacco R, Sorlie P, Stafford R, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J, Committee obotAHAS, Stroke Statistics Subcommittee. Heart disease and stroke statistics--2010 update: A report from the american heart association. *Circulation*. 2010;121:e46-215
14. World health organization. The top 10 causes of death. January 2017.

CONFIDENTIAL

15. Kathiresan S, Manning AK, Demissie S, D'Agostino RB, Surti A, Guiducci C, Gianniny L, Burt NP, Melander O, Orho-Melander M, Arnett DK, Peloso GM, Ordovas JM, Cupples LA. A genome-wide association study for blood lipid phenotypes in the framingham heart study. *BMC Medical Genetics*. 2007;8:S17-S17
16. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC, Jr., Watson K, Wilson PW. 2013 acc/aha guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: A report of the american college of cardiology/american heart association task force on practice guidelines. *J Am Coll Cardiol*. 2014;63:2889-2934
17. Fruchart JC, Davignon J, Hermans MP, Al-Rubeaan K, Amarenco P, Assmann G, Barter P, Betteridge J, Bruckert E, Cuevas A, Farnier M, Ferrannini E, Fioretto P, Genest J, Ginsberg HN, Gotto AM, Jr., Hu D, Kadowaki T, Kodama T, Krempf M, Matsuzawa Y, Nunez-Cortes JM, Monfil CC, Ogawa H, Plutzky J, Rader DJ, Sadikot S, Santos RD, Shlyakhto E, Sritara P, Sy R, Tall A, Tan CE, Tokgozo Lu L, Toth PP, Valensi P, Wanner C, Zambon A, Zhu J, Zimmet P. Residual macrovascular risk in 2013: What have we learned? *Cardiovasc Diabetol*. 2014;13:26
18. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (jelis): A randomised open-label, blinded endpoint analysis. *The Lancet*. 2007;369:1090-1098
19. Ginsberg HN, Elam MB, Lovato LC, Crouse JR, 3rd, Leiter LA, Linz P, Friedewald WT, Buse JB, Gerstein HC, Probstfield J, Grimm RH, Ismail-Beigi F, Bigger JT, Goff DC, Jr., Cushman WC, Simons-Morton DG, Byington RP. Effects of combination lipid therapy in type 2 diabetes mellitus. *N Engl J Med*. 2010;362:1563-1574
20. Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W. Niacin in patients with low hdl cholesterol levels receiving intensive statin therapy. *N Engl J Med*. 2011;365:2255-2267
21. Hps2-thrive randomized placebo-controlled trial in 25 673 high-risk patients of er niacin/laropiprant: Trial design, pre-specified muscle and liver outcomes, and reasons for stopping study treatment. *Eur Heart J*. 2013
22. Karlson BW, Wiklund O, Palmer MK, Nicholls SJ, Lundman P, Barter PJ. Variability of low-density lipoprotein cholesterol response with different doses of atorvastatin, rosuvastatin, and simvastatin: Results from voyager. *European heart journal. Cardiovascular pharmacotherapy*. 2016;2:212-217
23. Ridker PM, Mora S, Rose L. Percent reduction in ldl cholesterol following high-intensity statin therapy: Potential implications for guidelines and for the prescription of emerging lipid-lowering agents. *Eur Heart J*. 2016;37:1373-1379
24. Barber MJ, Mangravite LM, Hyde CL, Chasman DI, Smith JD, McCarty CA, Li X, Wilke RA, Rieder MJ, Williams PT, Ridker PM, Chatterjee A, Rotter JI, Nickerson DA, Stephens M, Krauss RM. Genome-wide association of lipid-lowering response to statins in combined study populations. *PLoS ONE*. 2010;5:e9763
25. Chasman DI, Giulianini F, MacFadyen J, Barratt BJ, Nyberg F, Ridker PM. Genetic determinants of statin-induced low-density lipoprotein cholesterol reduction: The justification for the use of statins in prevention: An intervention trial evaluating rosuvastatin (jupiter) trial. *Circ Cardiovasc Genet*. 2012;5:257-264
26. Hopewell JC, Parish S, Offer A, Link E, Clarke R, Lathrop M, Armitage J, Collins R. Impact of common genetic variation on response to simvastatin therapy among 18 705 participants in the heart protection study. *Eur Heart J*. 2013;34:982-992

CONFIDENTIAL

27. Postmus I, Trompet S, Deshmukh HA, Barnes MR, Li X, Warren HR, Chasman DI, Zhou K, Arsenault BJ, Donnelly LA, Wiggins KL, Avery CL, Griffin P, Feng Q, Taylor KD, Li G, Evans DS, Smith AV, de Keyser CE, Johnson AD, de Craen AJ, Stott DJ, Buckley BM, Ford I, Westendorp RG, Slagboom PE, Sattar N, Munroe PB, Sever P, Poulter N, Stanton A, Shields DC, O'Brien E, Shaw-Hawkins S, Chen YD, Nickerson DA, Smith JD, Dube MP, Boekholdt SM, Hovingh GK, Kastelein JJ, McKeigue PM, Betteridge J, Neil A, Durrington PN, Doney A, Carr F, Morris A, McCarthy MI, Groop L, Ahlqvist E, Bis JC, Rice K, Smith NL, Lumley T, Whitsel EA, Sturmer T, Boerwinkle E, Ngwa JS, O'Donnell CJ, Vasan RS, Wei WQ, Wilke RA, Liu CT, Sun F, Guo X, Heckbert SR, Post W, Sotoodehnia N, Arnold AM, Stafford JM, Ding J, Herrington DM, Kritchevsky SB, Eiriksdottir G, Launer LJ, Harris TB, Chu AY, Giulianini F, MacFadyen JG, Barratt BJ, Nyberg F, Stricker BH, Uitterlinden AG, Hofman A, Rivadeneira F, Emilsson V, Franco OH, Ridker PM, Gudnason V, Liu Y, Denny JC, Ballantyne CM, Rotter JI, Adrienne Cupples L, Psaty BM, Palmer CN, Tardif JC, Colhoun HM, Hitman G, Krauss RM, Wouter Jukema J, Caulfield MJ. Pharmacogenetic meta-analysis of genome-wide association studies of ldl cholesterol response to statins. *Nature communications*. 2014;5:5068
28. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: An integrative view. *Cell*. 2012;148:1258-1270
29. Ranjan R, Rani A, Metwally A, McGee HS, Perkins DL. Analysis of the microbiome: Advantages of whole genome shotgun versus 16s amplicon sequencing. *Biochem Biophys Res Commun*. 2016;469:967-977
30. Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. *Nat Biotechnol*. 2017;35:833-844
31. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006;444:1027-1031
32. Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, Chervonsky AV. Innate immunity and intestinal microbiota in the development of type 1 diabetes. *Nature*. 2008;455:1109-1113
33. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A*. 2004;101:15718-15723
34. Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc Natl Acad Sci U S A*. 2006;103:12511-12516
35. Kaddurah-Daouk R, Baillie RA, Zhu H, Zeng ZB, Wiest MM, Nguyen UT, Wojnoonski K, Watkins SM, Trupp M, Krauss RM. Enteric microbiome metabolites correlate with response to simvastatin treatment. *PLoS One*. 2011;6:e25482
36. Maurice CF, Haiser HJ, Turnbaugh PJ. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell*. 2013;152:39-50
37. Saha JR, Butler VP, Jr., Neu HC, Lindenbaum J. Digoxin-inactivating bacteria: Identification in human gut flora. *Science*. 1983;220:325-327
38. Islam KBMS, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, Ogura Y, Hayashi T, Yokota A. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology*. 2011;141:1773-1781
39. Joyce SA, MacSharry J, Casey PG, Kinsella M, Murphy EF, Shanahan F, Hill C, Gahan CGM. Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. *Proceedings of the National Academy of Sciences*. 2014;111:7421-7426

40. Yokota A, Fukiya S, Islam KBMS, Ooka T, Ogura Y, Hayashi T, Hagio M, Ishizuka S. Is bile acid a determinant of the gut microbiota on a high-fat diet? *Gut Microbes*. 2012;3:455-459
41. Donia MS, Fischbach MA. Human microbiota. Small molecules from the human microbiota. *Science*. 2015;349:1254766
42. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U S A*. 2009;106:3698-3703
43. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011;472:57-63
44. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WH, Bushman FD, Lusis AJ, Hazen SL. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med*. 2013;19:576-585
45. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, Li L, Fu X, Wu Y, Mehrabian M, Sartor RB, McIntyre TM, Silverstein RL, Tang WH, DiDonato JA, Brown JM, Lusis AJ, Hazen SL. Gut microbial metabolite tmao enhances platelet hyperreactivity and thrombosis risk. *Cell*. 2016;165:111-124
46. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. 2013;368:1575-1584
47. Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M, Culley MK, DiDonato AJ, Fu X, Hazen JE, Krajcik D, DiDonato JA, Lusis AJ, Hazen SL. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell*. 2015;163:1585-1595
48. Gérard P. Metabolism of cholesterol and bile acids by the gut microbiota. *Pathogens*. 2014;3
49. Ridlon JM, Kang D-J, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *Journal of Lipid Research*. 2006;47:241-259
50. Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R, Wilson-Perez HE, Sandoval DA, Kohli R, Backhed F, Seeley RJ. Fxr is a molecular target for the effects of vertical sleeve gastrectomy. *Nature*. 2014;509:183-188
51. Begley M, Gahan CG, Hill C. The interaction between bacteria and bile. *FEMS microbiology reviews*. 2005;29:625-651
52. Reihner E, Rudling M, Stahlberg D, Berglund L, Ewerth S, Bjorkhem I, Einarsson K, Angelin B. Influence of pravastatin, a specific inhibitor of hmg-coa reductase, on hepatic metabolism of cholesterol. *N Engl J Med*. 1990;323:224-228
53. Smith JL, Roach PD, Wittenberg LN, Riottot M, Pillay SP, Nestel PJ, Nathanson LK. Effects of simvastatin on hepatic cholesterol metabolism, bile lithogenicity and bile acid hydrophobicity in patients with gallstones. *Journal of gastroenterology and hepatology*. 2000;15:871-879
54. Parker RA, Garcia R, Ryan CS, Liu X, Shipkova P, Livanov V, Patel P, Ho SP. Bile acid and sterol metabolism with combined hmg-coa reductase and pcsk9 suppression. *J Lipid Res*. 2013;54:2400-2409
55. Jones ML, Martoni CJ, Prakash S. Cholesterol lowering and inhibition of sterol absorption by lactobacillus reuteri ncimb 30242: A randomized controlled trial. *Eur J Clin Nutr*. 2012;66:1234-1241
56. Jones BV, Begley M, Hill C, Gahan CG, Marchesi JR. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc Natl Acad Sci U S A*. 2008;105:13580-13585
57. Porez G, Prawitt J, Gross B, Staels B. Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease. *J Lipid Res*. 2012;53:1723-1737

CONFIDENTIAL

58. Ryan PM, Stanton C, Caplice NM. Bile acids at the cross-roads of gut microbiome-host cardiometabolic interactions. *Diabetol Metab Syndr*. 2017;9:102
59. Kliewer SA, Mangelsdorf DJ. Bile acids as hormones: The fxr-fgf15/19 pathway. *Digestive diseases (Basel, Switzerland)*. 2015;33:327-331
60. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor fxr/bar impairs bile acid and lipid homeostasis. *Cell*. 2000;102:731-744
61. Angelin B, Larsson TE, Rudling M. Circulating fibroblast growth factors as metabolic regulators--a critical appraisal. *Cell Metab*. 2012;16:693-705
62. Lundasen T, Galman C, Angelin B, Rudling M. Circulating intestinal fibroblast growth factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man. *J Intern Med*. 2006;260:530-536
63. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarthy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E. Farnesoid x nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (flint): A multicentre, randomised, placebo-controlled trial. *Lancet*. 2015;385:956-965
64. Olsson AG, Pears J, McKellar J, Mizan J, Raza A. Effect of rosuvastatin on low-density lipoprotein cholesterol in patients with hypercholesterolemia. *Am J Cardiol*. 2001;88:504-508
65. McKenney JM. Efficacy and safety of rosuvastatin in treatment of dyslipidemia. *American Journal of Health-System Pharmacy*. 2005;62:1033
66. Rosuvastatin (crestor) [us prescribing information], astrazeneca pharmaceuticals, wilmington, de, 2018.
67. Ridker PM, Danielson E, Fonseca FAH, Genest J, Gotto AMJ, Kastelein JJP, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ. Rosuvastatin to prevent vascular events in men and women with elevated c-reactive protein. *New England Journal of Medicine*. 2008;359:2195-2207
68. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. *Science (New York, N.y.)*. 2011;334:105-108
69. Park Y, Dodd KW, Kipnis V, Thompson FE, Potischman N, Schoeller DA, Baer DJ, Midthune D, Troiano RP, Bowles H, Subar AF. Comparison of self-reported dietary intakes from the automated self-administered 24-h recall, 4-d food records, and food-frequency questionnaires against recovery biomarkers. *Am J Clin Nutr*. 2018;107:80-93
70. Edgar RC. Search and clustering orders of magnitude faster than blast. *Bioinformatics*. 2010;26:2460-2461
71. Lozupone C, Knight R. Unifrac: A new phylogenetic method for comparing microbial communities. *Applied and environmental microbiology*. 2005;71:8228-8235
72. Lozupone CA, Hamady M, Kelley ST, Knight R. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Applied and environmental microbiology*. 2007;73:1576-1585
73. O'Toole PW, Jeffery IB. Gut microbiota and aging. *Science*. 2015;350:1214
74. Tang WH, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. *Circ Res*. 2017;120:1183-1196
75. Li M, Wang B, Zhang M, Rantalainen M, Wang S, Zhou H, Zhang Y, Shen J, Pang X, Zhang M, Wei H, Chen Y, Lu H, Zuo J, Su M, Qiu Y, Jia W, Xiao C, Smith LM, Yang S, Holmes E, Tang H, Zhao G, Nicholson JK, Li L, Zhao L. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc Natl Acad Sci U S A*. 2008;105:2117-2122

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76. National cancer institute. Asa24. Available from: <https://epi.Grants.Cancer.Gov/asa24/> (accessed 8/5/2020).
77. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. Qiime allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010;7:335-336
78. McKenna P, Hoffmann C, Minkah N, Aye PP, Lackner A, Liu Z, Lozupone CA, Hamady M, Knight R, Bushman FD. The macaque gut microbiome in health, lentiviral infection, and chronic enterocolitis. *PLoS pathogens*. 2008;4:e20
79. Wu GD, Lewis JD, Hoffmann C, Chen YY, Knight R, Bittinger K, Hwang J, Chen J, Berkowsky R, Nessel L, Li H, Bushman FD. Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16s sequence tags. *BMC Microbiol*. 2010;10:206
80. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. An improved greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME journal*. 2012;6:610-618
81. Price MN, Dehal PS, Arkin AP. Fasttree 2--approximately maximum-likelihood trees for large alignments. *PLoS One*. 2010;5:e9490
82. Segata N, Waldron L, Ballarini A, Narasimhan V, Jousson O, Huttenhower C. Metagenomic microbial community profiling using unique clade-specific marker genes. *Nat Methods*. 2012;9:811-814
83. Kanehisa M, Goto S. Kegg: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28:27-30
84. Wu GD, Compher C, Chen EZ, Smith SA, Shah RD, Bittinger K, Chehoud C, Albenberg LG, Nessel L, Gilroy E, Star J, Weljie AM, Flint HJ, Metz DC, Bennett MJ, Li H, Bushman FD, Lewis JD. Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. *Gut*. 2016;65:63-72
85. Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*. 2001;26:32-46
86. Zhao N, Chen J, Carroll IM, Ringel-Kulka T, Epstein MP, Zhou H, Zhou JJ, Ringel Y, Li H, Wu MC. Testing in microbiome-profiling studies with mirkat, the microbiome regression-based kernel association test. *Am J Hum Genet*. 2015;96:797-807
87. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepille DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C. Predictive functional profiling of microbial communities using 16s rRNA marker gene sequences. *Nature Biotechnology*. 2013;31:814
88. Abubucker S, Segata N, Goll J, Schubert AM, Izard J, Cantarel BL, Rodriguez-Mueller B, Zucker J, Thiagarajan M, Henrissat B, White O, Kelley ST, Methe B, Schloss PD, Gevers D, Mitreva M, Huttenhower C. Metabolic reconstruction for metagenomic data and its application to the human microbiome. *PLoS Comput Biol*. 2012;8:e1002358
89. Caspi R, Billington R, Ferrer L, Foerster H, Fulcher CA, Keseler IM, Kothari A, Krummenacker M, Latendresse M, Mueller LA, Ong Q, Paley S, Subhraveti P, Weaver DS, Karp PD. The metacyc database of metabolic pathways and enzymes and the biocyc collection of pathway/genome databases. *Nucleic Acids Res*. 2016;44:D471-480
90. Chen EZ, Li H. A two-part mixed-effects model for analyzing longitudinal microbiome compositional data. *Bioinformatics*. 2016;32:2611-2617

**UNIVERSITY OF PENNSYLVANIA****Combined Consent and HIPAA Authorization to Participate as a Subject in an Research Study**

**Protocol Title:** Investigation of the Gut microbiome on Satin response (INGEST)

**Principal Investigator:** Sony Tuteja, PharmD, MS  
Smilow Center for Translational Research 11-143  
215-573-7834

**Emergency Contact:** Sony Tuteja, PharmD, MS  
484-431-1002 (cell) or

Archna Bajaj, MD  
518-421-1996 (cell)

**Summary**

You are being invited to participate in a research study. Your participation is voluntary and you should only participate if you completely understand what the study requires and what the risks of participation are. You should ask the study team any questions you have related to participating before agreeing to join the study. If you have any questions about your rights as a human research participant at any time before, during or after participation, please contact the Institutional Review Board (IRB) at (215) 898-2614 for assistance.

The research study is being conducted to determine if statin medications impact the composition of the bacteria that reside in our gut; and if the gut bacteria affects response to statin medications. This study is being conducted in healthy volunteers and you are eligible for participation because you are in overall good health.

If you agree to join the study, you will be asked to complete the following research procedures:

- Take a rosuvastatin or placebo pill daily for 8 weeks
- Provide blood (4), stool (3) and urine (3) samples for laboratory measures and microbiome analyses
- Complete food recall questionnaires describing your 24 hour food intake
- Undergo a medical examination
- Participate in a phone call check-in with study staff after 4 weeks of taking the study drug/placebo

Your participation will last for 16 weeks.

You are not expected to receive any benefit from being in this research study except for the knowledge that you are participating in research that could help researchers understand how the gut bacteria affect response to medications.

The most common risks related to the study medication include: headache, mild muscle aches or pains, constipation, nausea, and diarrhea. In rare cases, the study drug may cause: fever, unusual tiredness, dark colored urine; severe muscle pain, tenderness or weakness; confusion, memory problems and the drug will be stopped. Blood draws may be associated with bleeding, infection and bruising at the access site. Additionally, there is the risk of loss of privacy.

Please note that there are other factors to consider before agreeing to participate such as additional procedures, use of your personal information, costs, and other possible risks not discussed here. If you are interested in participating, a member of the study team will review the full information with you. You are free to decline or stop participation at any time during or after the initial consenting process.

### **Why am I being asked to volunteer?**

You are being invited to participate in a research study because you are a healthy adult. Your participation is voluntary which means you can choose whether or not you want to participate. If you choose not to participate, there will be no loss of benefits to which you are otherwise entitled. Before you can make your decision, you will need to know what the study is about, the possible risks and benefits of being in this study, and what you will have to do in this study. The research team is going to talk to you about the research study, and they will give you this consent form to read. You may also decide to discuss it with your family, friends, or family doctor. You may find some of the medical language difficult to understand. Please ask the investigator and/or the research team about anything you do not understand. If you decide to participate, you will be asked to sign this form

### **What is the purpose of this research study?**

There is evidence that the bacteria that naturally reside in our gut can influence how well we respond to medications. Therefore this study will look at how rosuvastatin, a medication used to lower cholesterol levels, may change the bacteria in the gut. We will also examine how the gut bacteria will affect the ability of rosuvastatin to lower cholesterol levels.

### **How long will I be in the study?**

If you consent to participate, you will participate for 16 weeks. This includes the time that may lapse between your screening visit (today) and the day you start the study (Visit 1.) There will be approximately 8 weeks between Visits 1 and 2, and approximately 4 weeks between Visits 2 and 3.

The expected duration of the study is 2 years. We plan to enroll 100 participants during that time.

## What am I being asked to do?

After you consent to participate, we will complete this screening visit to determine your eligibility to participate. At this screening we will: fill out a medical history questionnaire that will include demographic information, complete a physical exam (including measuring your vitals and height and weight), draw up to 3 tubes of blood (equal to 6 teaspoons), and do a urine pregnancy test for females. Some visit activities may be completed remotely by phone, email, or telemedicine.

If you are determined to be eligible to participate after the screening visit, you will be scheduled to come to the Center for Human Phenomic Sciences at the hospital for 3 visits. Below is a list of what you will be asked to do each day you come in:

- Physical exam – once on day 1 (screening visit)
- Medical history – once on day 1 – complete a questionnaire about your medical history (screening visit)
- Diet recall – prior to Visits 1, 2 and 3 – complete a 24-hour food recall for 3 days prior to Visits 1 and 2, and for 1 day prior to Visit 3.
- Stool collection at home and bring to each of the remaining 3 study visits.
- Blood draw – on each of the 4 study visits. Up to 30 milliliters of blood will be drawn on these days, equal to about 6 teaspoons.
- Urine sample- on visit 1, 2, and 3.
- Take a study medication once per day for 8 weeks (see below).
- We will store blood, urine, stool and DNA samples for future research.
- You will have a phone call check-in with study staff between visits 1 and 2

Study medication. You will be randomized to one of two study medications: Rosuvastatin 10 or 20 mg daily by mouth for 8 weeks or a placebo (sugar pill). You will not know which treatment you will be receiving. Rosuvastatin 10 or 20 mg is approved by the Food and Drug Administration for the treatment of high cholesterol.

## What are the possible risks or discomforts?

If you experience any risks or discomforts while in this study, please let the study doctor know immediately using the emergency contact information on page one of this form.

### Rosuvastatin

The most common side effects reported with rosuvastatin include headache, mild muscle aches or pains, constipation, nausea, and diarrhea. Some side effects can be serious. The following symptoms are uncommon but more serious: fever, unusual tiredness, dark colored urine; severe muscle pain, tenderness or weakness; confusion, memory problems.

### Blood draw

There are a small number of risks associated with drawing blood. You may develop a bruise or bump at the site of the needle puncture. Some individuals get light-headed

and on rare occasions faint when blood is drawn. Another risk of drawing blood is the potential for infection, which is very rare, and/or pain at the site of the needle puncture.

**Reproductive risks:** Because of the effects of this drug, there could be serious harm to unborn children or children who are breast-feeding. These effects could also harm the mother. It is also possible that harmful side effects that are not yet known could happen to both the mother and unborn or breast-feeding child. If you are currently pregnant, it is important that you inform the investigator because you will not be able to participate in the study. If you are able to become pregnant, you will be given a urine pregnancy test before entry into the study. You are asked to use two medically accepted methods of birth control (e.g. combined oral contraceptives, barrier methods, approved contraceptive implant, long-term injectable contraception, or intrauterine device) for the entire duration of the study. You should not become pregnant while you are taking this drug. If you do become pregnant, you must tell the investigator and consult an obstetrician or maternal-fetal specialist.

You may be exposed to some **non-physical risks**:

- Some questions that we ask in the interviews may make you feel uncomfortable. You can refuse to answer any question, but in some cases, this may mean that we will not be able to use your samples for the study.
- All research studies are associated with a possible loss of confidentiality but we will do everything we can to protect your information and confidentiality.

There may be unforeseeable risks at this time. Should we find out about new risks we will inform you immediately.

### **What if new information becomes available about the study?**

During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about being in the study. We will notify you as soon as possible if such information becomes available.

### **What are the possible benefits of the study?**

You are not expected to receive any benefit from being in this research study except for the knowledge that you are participating in research that could help researchers understand how the gut bacteria affect response to medications.

### **What other choices do I have if I do not participate?**

The alternative to participating is to not participate. If you decide not to participate, it will not affect:

- the treatment or care your health care providers give you;
- your insurance payments or enrollment in any health plans; or
- any benefits to which you are otherwise entitled.

**Will I be paid for being in this study?**

You will be compensated \$225 for completion of the study. You will receive \$75 for visit 1, \$75 for visit 2, and \$75 for visit 3. If needed, you will also be provided with parking vouchers on the day of the study visits.

Please note: In order to be compensated for your participation in this study, you must provide your Social Security Number. Additionally, please note that the University of Pennsylvania is required to report to the IRS any cumulative payments for participation in research studies that exceed a total of \$600 in a calendar year.

**Will I have to pay for anything?**

All of the study procedures and tests are covered by the study and you will not have to pay for anything.

You and/or your health insurance may be billed for the costs of medical care during this study if these expenses would have happened even if you were not in the study, or if your health insurance company agrees in advance to pay these costs.

**What happens if I am injured from being in the study?**

We will offer you the care needed to treat injuries directly resulting from taking part in this research. We may bill your insurance company or other third parties, if appropriate, for the costs of the care you get for the injury, but you may also be responsible for some of them.

There are no plans for the University of Pennsylvania to pay you or give you other compensation for the injury. You do not give up your legal rights by signing this form.

If you think you have been injured as a result of taking part in this research study, tell the person in charge of the research study as soon as possible. The researcher's name and phone number are listed in the consent form.

**When is the Study over? Can I leave the Study before it ends?**

This study is expected to end after all participants have completed all visits, and all information has been collected. This study may also be stopped at any time by your physician, the study Sponsor, or the Food and Drug Administration (FDA) without your consent because:

- The Primary Investigator feels it is necessary for your health or safety. Such an action would not require your consent, but you will be informed if such a decision is made and the reason for this decision.
- You have not followed study instructions.
- The Sponsor, the study Principal Investigator, or the Food and Drug Administration (FDA) has decided to stop the study.

If you decide to participate, you are free to leave the study at anytime. Withdrawal will not interfere with your future care.

## **What information about me may be collected?**

The following personal health information may be collected, used for research and may be disclosed or released during your involvement with this research study:

- Name
- Address
- Telephone number
- Email address
- Medical Record Number
- Social security number (used to provide compensation for the study)
- Allergies
- Family Medical history
- Information from past and current medical and medicine history
- Information from tests and procedures

## **Why is my information being used?**

Your information is used by the research team to contact you during the study. Your information and results of test and procedures are used to:

- Do the research
- Communicate to you about the research
- Oversee the research
- See if the research was done right.

## **Who may use and share information about me?**

The following individuals may use or share your information for this research study:

- The Principal Investigator and the Investigator's study team (other University staff associated with the study)
- The University of Pennsylvania Institutional Review Boards (the committees charged with overseeing research on human subjects) and University of Pennsylvania Office of Regulatory Affairs
- The University of Pennsylvania Office of Human Research (the office which monitors research studies)
- Authorized members of the University of Pennsylvania and the University of Pennsylvania Health System and School of Medicine who may need to access your information in the performance of their duties (for example: to provide treatment, to ensure integrity of the research, accounting or billing matters, etc).

## **Who, outside the School of Medicine, might receive my information?**

Researchers from outside the University of Pennsylvania may receive past and current medical and medicine history and information from tests acquired during this research study. Researchers from outside the University will not receive your name, address, telephone number, email address, medical record number or social security number. Once your personal health information listed above is disclosed to others outside the School of Medicine, it may no longer be covered by federal privacy protection regulations.

### **How long may the School of Medicine use or disclose my personal health information?**

Your authorization for use of your personal health information for this specific study does not expire. Your information may be held in a research database. However, the School of Medicine may not re-use or re-disclose information collected in this study for a purpose other than this study unless:

- You have given written authorization
- The University of Pennsylvania's Institutional Review Board grants permission
- As permitted by law

### **Can I change my mind about giving permission for use of my information?**

Yes. You may withdraw or take away your permission to use and disclose your health information at any time. You do this by sending written notice to the investigator for the study. If you withdraw your permission, you will not be able to stay in this study.

### **What if I decide not to give permission to use and give out my health information?**

Then you will not be able to be in this research study.

### **How will my personal information be protected during the study?**

We will do our best to make sure that the personal information obtained during the course of this research study will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. The Institutional Review Board (IRB) at the University of Pennsylvania will have access to your records.

The data and samples that will be analyzed will be de-identified meaning that those people who are studying your samples and data will not know that the samples and data come from you. Instead, data and samples will be labeled with a unique study identification number and only certain members of the study team will have access to the key to these study identification numbers.

### **Will information about this study be available to the public?**

A description of this clinical trial will be available on [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov), as required by U.S. Law. This website will not include information that can identify you.

### **What may happen to my information and samples collected on this study?**

Your information and samples will be de-identified. De-identified means that all identifiers have been removed. The information and samples could be stored and shared for future research in this de-identified fashion. It would not be possible for future researchers to identify you as we would not share any identifiable information about you with future researchers. This can be done without again seeking your consent in the

future, as permitted by law. The future use of your information and samples only applies to the information and samples collected on this study. Your information and samples may be stored and used for future research purposes for an indefinite amount of time. There are no plans to tell you about any of the specific research that will be done. Possible future research may include identifying new proteins or molecules in your samples that are helpful to predict response to medications. We may share your de-identified information and samples with other research, academic and medical institutions, other researchers, drug and device companies, biotechnology companies and others. We will not follow up with you to tell you about the specific research that will be done. We will not give you any results from these studies. It is possible that you may have chosen not to participate in these future research studies, had you been approached for participation.

There is a risk of breach of confidentiality (unintentional release of your information). We will do our best to make sure that this doesn't happen. However, we cannot guarantee total privacy. We will protect your confidentiality during storage and sharing by storing your information in a pass-word protected database and not using personal identifiers with the sample.

You will likely not directly benefit from future research with your information and samples. Research with your identifiable information and samples may help others by improving our understanding of health and disease, improving health care and making safer or more effective medical therapies, and developing new scientific knowledge.

If you have questions about the storage of your information and samples, or have changed your mind, you can contact Sony Tuteja at 215-573-7834. If you change your mind, your sample and data will be removed from storage. If analysis was already performed with your sample then we will use the data generated from your sample but we will not perform additional analysis on your sample.

Your samples will not be sold to any person, institution or company for financial gain or commercial profit. Neither you nor your heirs will gain financially from discoveries made this the information and/or specimens that you provide.

## ***Electronic Medical Records and Research Results***

### **What is an Electronic Medical Record and/or a Clinical Trial Management System?**

An Electronic Medical Record (EMR) is an electronic version of the record of your care within a health system. An EMR is simply a computerized version of a paper medical record.

A clinical trial management system (CTMS) is used to register your information as a participant in a study and to allow for your research data to be entered/stored for the purposes of data analysis and any other required activity for the purpose of the conduct of the research.

If you are receiving care or have received care within the University of Pennsylvania Health System (UPHS) (outpatient or inpatient) and are participating in a University of Pennsylvania research study, information related to your participation in the research (i.e. laboratory tests, imaging studies and clinical procedures) may be placed in your existing EMR maintained by UPHS. Information related to your participation in clinical research will also be contained in the CTMS.

If you have never received care within UPHS and are participating in a University of Pennsylvania research study that uses UPHS services, an EMR will be created for you for the purpose of maintaining any information produced from your participation in this research study. The creation of this EMR is required for your participation in this study. In order to create your EMR, the study team will need to obtain basic information about you that would be similar to the information you would provide the first time you visit a hospital or medical facility (i.e. your name, the name of your primary doctor, the type of insurance you have). Information related to your participation in the study (i.e. laboratory tests, imaging studies and clinical procedures) may be placed in this EMR. Once placed in your EMR or in the CTMS, your information may be accessible to appropriate UPHS workforce members that are not part of the research team. Information within your EMR may also be shared with others who are determined by UPHS to be appropriate to have access to your EMR (e.g. Health Insurance Company, disability provider, etc.).

### **Who can I call with questions, complaints or if I'm concerned about my rights as a research subject?**

If you have questions, concerns or complaints regarding your participation in this research study or if you have any questions about your rights as a research subject, you should speak with the Principal Investigator listed on page one of this form. If a member of the research team cannot be reached or you want to talk to someone other than those working on the study, you may contact the Office of Regulatory Affairs with any question, concerns or complaints at the University of Pennsylvania by calling (215) 898-2614.

When you sign this form, you are agreeing to take part in this research study. This means that you have read the consent form, your questions have been answered, and you have decided to volunteer. Your signature also means that you are permitting the University of Pennsylvania to use your personal health information collected about you for research purposes within our institution. You are also allowing the University of Pennsylvania to disclose that personal health information to outside organizations or people involved with the operations of this study.

A copy of this consent form will be given to you.

|                                         |                               |               |
|-----------------------------------------|-------------------------------|---------------|
| _____<br>Name of Subject (Please Print) | _____<br>Signature of Subject | _____<br>Date |
|-----------------------------------------|-------------------------------|---------------|

|                                                             |                    |               |
|-------------------------------------------------------------|--------------------|---------------|
| _____<br>Name of Person Obtaining<br>Consent (Please Print) | _____<br>Signature | _____<br>Date |
|-------------------------------------------------------------|--------------------|---------------|

### **Pharmacokinetic substudy (OPTIONAL)**

Approximately 40 participants in this study will be randomly selected to participate in an optional pharmacokinetic study. In place of Visit 2, participants will be scheduled for a 12 hours visit at the Center for Human Phenomic Sciences at the hospital. Twelve blood samples will be obtained (approximately 8 teaspoons) over the 12 hours. Three meals and one snack will be provided during this day. Concentrations of the study medication will be determined from the blood samples. You will be compensated an additional \$100 for this portion of the study.

**Choose one of the following options by initialing the line:**

\_\_\_\_\_ I am interested in participating in this substudy.

\_\_\_\_\_ I am not interested in participating in this substudy.

### **Future Research (OPTIONAL)**

We would like your permission to perform genetic tests related to cardiovascular disease and medication response on the blood sample we have collected. If you give us permission your blood will be tested for many genes related to cardiovascular disease, diabetes, or response to medications. Your blood specimen will be given a special coded number and will not contain your name or any other identifying information about you. You may still participate in the current study even if you would not like to give us permission for future genetic tests. Please read the following two statements listed below and initial one of the lines indicating if you would like to provide authorization for future genetic tests related to cardiovascular disease.

**Choose one of the following options by initialing the line:**

\_\_\_\_\_ I give you permission to perform genetic tests related to cardiovascular disease on the sample I have already provided.

\_\_\_\_\_ I do not give you permission to perform additional genetic tests related to cardiovascular disease on the sample I have already provided.

### **What should I know about the genetic testing being done in this study?**

This optional research includes genetic testing. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information. Whole genome sequencing will not be conducted on your samples. Whole genome sequencing involves analyzing your entire personal genetic code.

Your personal information (name, address, telephone number, date of birth) will not be attached to the blood used for genetic research. The DNA and all results of genetic testing will be stored under a code number without any information that may identify

you, a secure password-protected database. The researchers who view this database will not have access to your name or identifying information. The results of the genetic analysis are for research purposes only and will be kept confidential.

There can be a risk in knowing genetic information. New health information about inherited traits that might affect you or your blood relatives could be found during a research study. Even though your genes are unique, you share some of the same genes with your blood relatives. Although we are not able to know all of the risks from taking part in research on inherited traits, we believe that the risks to you and your family are very low, because your samples will be coded. Research results will not be returned to you or your doctor.

Very rarely health or genetic information could be misused by employers, insurance companies, and others. For example, it could make it harder for you to get or keep a job or insurance, or life insurance companies may charge a higher rate based on this information. We believe the chance these things will happen is very small, but we cannot make guarantees.

A recent federal law (Genetic Information Non-Discrimination Act, GINA) helps reduce the risk from health insurance or employment discrimination. The law does not include other types of misuse by life insurance or long term care insurance. If you want to learn more about GINA, you can find information on the internet (<https://www.hhs.gov/hipaa/for-professionals/special-topics/genetic-information/index.html>) or ask the study staff.

In addition, information from this study may lead to new questions that may result in either new research studies. Please indicate below if we may contact you about studies in which you may qualify. Any new information will be kept in a secure research database as described above

**Choose one of the following options by initialing the line:**

\_\_\_\_\_ You may contact me in the future about studies for which I may qualify

\_\_\_\_\_ You may not contact me in the future about research studies for which I may qualify