Effects of Pitavastatin on Insulin Sensitivity and Liver Fat
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1. Background and Significance

HMG co-A reductase inhibitors, commonly called statins, are an effective treatment for dyslipidemia and atherosclerotic heart disease with proven mortality benefit. Cholesterol lowering drugs are currently used by 10.7% of the US population under age 64 years and by 46.7% of the population 65 years old and above (http://www.cdc.gov/nchs/data/hus/hus13.pdf; CDC Health United States Report 2013). Under recently published 2013 American College of Cardiology and American Heart Association (ACC-AHA) guidelines for cholesterol management[1], approximately 56 million US adults will be eligible for use of statins[2]. Although the lipid-lowering effects of statins are well-known, other metabolic effects, including effects on glucose tolerance and ectopic fat distribution, are less completely understood. It is increasingly clear that (i) statins have pleiotropic effects, and may confer metabolic and cardiovascular benefit through mechanisms beyond lipid lowering, and (ii) different statins may have dissimilar effects on non-lipid metabolic endpoints. For example, many statins appear to worsen glucose homeostasis[3], whereas others may be neutral in this regard. Additionally, some statins may have benefit in decreasing hepatic lipid accumulation and associated steatohepatitis[4]. Thus characterizing the metabolic effects of particular statins is critical to inform prescribers' choice of agent based on clinical context. Pitavastatin, FDA approved in 2009, is the most recent statin to enter the market. Previous studies have suggested that pitavastatin may be neutral to glucose homeostasis[5,6] and may improve hepatic lipid[7-9]. Neither of these effects has been proven definitively, however, and the current proposal aims to characterize in detail the effects of pitavastatin on glucose homeostasis, hepatic steatosis, and steatohepatitis.

Although statin use is generally safe, recent studies have demonstrated that statin use is associated with increased incidence of diabetes mellitus (DM). This association was first reported in The Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial in 2008[3], a study of 17,802 subjects in which rosuvastatin was found to be associated with an increase in DM incidence compared to placebo. Since the publication of the JUPITER trial, several meta-analyses and post-hoc analyses have been performed including one large meta-analyses comprising 13 statin trials and 91,140 patients without DM, demonstrating an overall 9% increased risk of incident DM over 4 years with statin use[10]. The diabetogenic effect of statins appears to be variable, with some statins confering greater risk than others. Generally, atorvastatin[11] and rosuvastatin[11,12] appear to confer the greatest risk while pravastatin[12,13] and pitavastatin[5,13-16] appear to confer the least risk for development of DM and may be neutral to glucose homeostasis. While this is encouraging for use and development of pitavastatin, the effects of pitavastatin on diabetes risk have not been studied in great detail.

In addition to its neutrality with respect to glucose homeostasis, pitavastatin may ameliorate hepatic steatosis and steatohepatitis. Nonalcoholic fatty liver disease (NAFLD) – defined as hepatic triglyceride content comprising ≥5% of total liver weight – is highly prevalent among the U.S. population and is a significant co-morbidity of obesity. Data from the Dallas Heart Study suggest a prevalence of NAFLD of approximately 33%[17]. The disease spectrum of NAFLD encompasses not only steatosis but also inflammation, hepatocellular damage, and fibrosis, which are hallmarks of non-alcoholic steatohepatitis (NASH). NASH, in turn, may progress to cirrhosis and eventual liver failure. NASH is also a significant risk factor for hepatocellular carcinoma [18]. NAFLD is also strongly associated with visceral adiposity and other

cardiometabolic abnormalities, including insulin resistance (IR) and cardiovascular disease (CVD) [19,20]. Abundant evidence suggests a causal link between NAFLD and IR, and emerging data suggest that NAFLD may also be causal in CVD[21-23]. Thus amelioration of NAFLD is a significant clinical priority both to preserve liver health and improve cardiometabolic risk.

Multiple studies have investigated the use of various statins for NAFLD and NASH, with the hypothesis that statins may prove beneficial due to lipid-lowering action as well as other pleiotropic effects such as reduction of inflammation[4]. These studies have shown mixed results, with many having limited validity due to small sample size and/or open-label design[4]. For pitavastatin in particular, however, animal data[7,24-26] and preliminary human studies[8,9] consistently suggest an effect to decrease liver fat content and ameliorate features of NASH. In murine models of NAFLD and NASH, pitavastatin consistently decreases steatosis, reduces transaminases, and ameliorates features of steatohepatitis[6,21-23]. The mechanisms of these effects may include (i) decreased lipogenesis and increased fatty acid oxidation[7,25], possibly through increased activation of hepatic AMPK-α[25,27] and/or PPARα[7]; (ii) reduced expression of inflammatory cytokines, including TNF-α and IL-6[24-27], and chemokine receptors, including CCR2[26,28] and CCR5[28]; (iii) reduced oxidative stress[24]; (iv) increased adiponectin[25,27]; and (v) reduced macrophage infiltration[26]. In open-label studies in humans, pitavastatin has shown some benefit to ameliorate NAFLD and NASH[8,9], but a randomized controlled trial has not yet been performed.

2. Specific Aims

In a randomized, placebo-controlled trial of 50 individuals receiving pitavastatin vs. identical placebo for six months, we will test the following hypotheses:

Specific Aim 1: Pitavastatin will not significantly differ from placebo with respect to changes in the following measures of glucose homeostasis:

a. Insulin-stimulated glucose uptake during standard euglycemic hyperinsulinemic clamp
b. Insulin suppression of hepatic glucose production during "low-dose" euglycemic hyperinsulinemic clamp
c. Fasting glucose, HOMA-IR, and HbA1c

Specific Aim 2: Pitavastatin will significantly reduce liver fat and improve markers of NAFLD.

a. Compared to placebo, pitavastatin will significantly reduce liver fat content as measured by ^{1}H-magnetic resonance spectroscopy (MRS).
b. In the subgroup of patients with ≥5% liver fat by 1H-MRS, compared to placebo, pitavastatin will significantly decrease AST and ALT.

3. Subject Selection

50 men will be recruited from the greater Boston area. The study is limited to men in order to increase the homogeneity of the subject population, particularly with respect to hormonal milieu and CVD risk given the substantial variance between men and women in these areas. Subjects will be recruited according to the following inclusion and exclusion criteria:

Inclusion Criteria

1. Men age 40-65yo
2. BMI ≥ 27 kg/m^2 and waist circumference ≥102cm, high probability risk factors for NAFLD
3. At least one of the following indicating insulin resistance: Fasting glucose ≥100mg/dL and <126mg/dL, HOMA-IR >2.0, and/or 2 hour glucose ≥140mg/dL and <200mg/dL following standard glucose tolerance test.

4. 10-year cardiovascular disease risk ≥5% by American Heart Association(AHA)/American College of Cardiology (ACC) Pooled Cohort Equations CV Risk Calculator[29] or LDL ≥ 100mg/dL
5. No use of any statin within 1 year of study entry and not being actively considered for statin therapy by a treating provider.

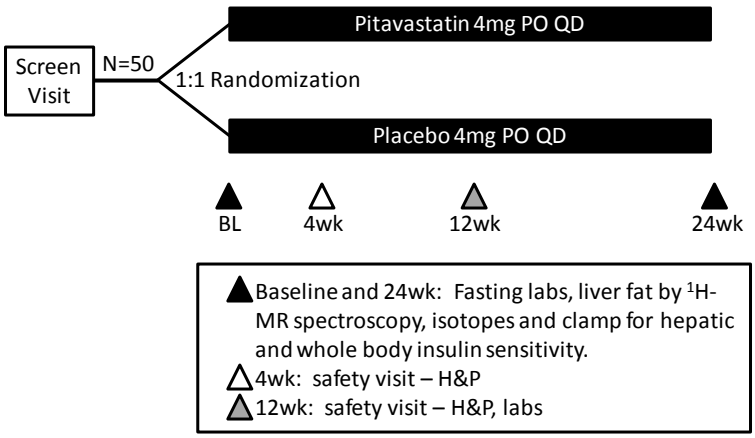
Exclusion Criteria
1. Diagnosis of diabetes or use of anti-diabetic medications.
2. Use of erythromycin, rifampin, cyclosporin, colchicine, or gemfibrozil.
3. Use of statin therapy within 1 year prior to study entry as above. Use of any other lipid-modifying therapy (including fish oil, fibrates, niacin, gemfibrozil) within 6 months of study entry.
4. Contraindication to statin therapy.
5. Creatinine > upper limit of normal or known renal disease
6. AST or ALT > 3 times the upper limit of normal
7. Hb < 10g/dL
8. Contraindication to undergoing a magnetic resonance scan.
9. Atherosclerotic cardiovascular disease or LDL-C ≥ 190mg/dL.
10. Triglyceride ≥500mg/dL

4. Subject Enrollment

Methods of Enrollment

The study will be advertised through ambulatory clinics affiliated with the Massachusetts General Hospital, through the MGH Research Study Volunteer Participant database and Partners Clinical Trials website, and through flyers and newspaper advertisements. Craigslist will also be utilized, with a link to a REDCap Survey that individuals can fill out. We will also place targeted advertisements on Facebook with links to the same REDCap Survey. This survey will collect minimal information necessary to see if subjects would potentially be eligible, as well as contact information so that we can call them if they are potentially eligible. We will also send targeted mailings to past participants of the SUGAR MGH and SIGMA studies who have indicated on written informed consent that they are willing to be contacted for future studies. We will also perform a Partners Research Patient Data Registry (RPDR) search to identify patients with BMI $\geq$ 27, who meet our age and sex criteria, who are not currently on diabetes or cholesterol medications. We will subsequently review the resulted medical records to hone in on those that meet our inclusion/exclusion criteria. We will **directly** contact **patients with a mailing who have agreed to be approached by research staff as part of the Research Opportunities Direct to You (RODY) Program.** Interested subjects will call and speak with study staff. Preliminary eligibility will be determined based on study staff interviews of interested subjects over the phone. Eligible subjects will then be scheduled for a screening visit. Potential participants who express interest in receiving study related materials and communications by e-mail (including a copy of the consent form) will be educated regarding the Partners HealthCare e-mail security policy. If the subject does not want to use encrypted email, they can consent to receive unencrypted e-mail, and this will be documented in the subject's record as per the subject's request.

Informed Consent
Written informed consent will be obtained by a licensed physician or nurse practitioner investigator prior to screening evaluation and testing. Subjects will be informed that they may withdraw from participation in the study at any point.



Randomization
After signing consent and, prior to the baseline visit, eligible subjects will be randomized to receive pitavastatin 4mg orally daily vs. identical placebo for 6 months. Randomization will be performed by the MGH Research Pharmacy and will be blinded to study investigators and subjects.

5. Study Procedures

This double-blind, randomized, placebo-controlled study will include a screening visit and, for eligible subjects, 4 additional visits over a 6 month treatment period. All subjects will receive lifestyle and nutrition counseling at the baseline, 12 week, and 24 week visits as per standard guidelines, including advice on caloric intake, salt, fiber, cholesterol, fat and exercise, and smoking cessation.

All subject encounters will take place at the MGH GCRC.

Screen Visit (to determine eligibility)
1. Informed consent
2. Detailed medical history and medications (past and current) and physical exam
3. Height, weight, waist and hip circumferences.
4. Fasting blood samples (CBC, Creatinine, ALT, AST, lipid panel, fasting glucose)
5. Standard 75g oral glucose tolerance test

Baseline Visit
1. Interval H&P
2. Fasting blood samples (Fasting glucose, insulin, HbA1c, ALT, AST, CPK, lipid panel and direct LDL, CoQ10, pro-insulin)
3. ^{1}H-MRS/MRI abdomen to quantify liver fat, visceral and subcutaneous adipose tissue (VAT and SAT)
4. Stable isotope infusion to quantify hepatic gluconeogenesis.
5. Two-step euglycemic hyperinsulinemic clamp to assess insulin sensitivity of liver (“low-dose” clamp) and muscle (standard dose clamp)
6. Whole body DXA scan to quantify total and regional fat and lean mass
7. 4-day food record and modifiable activity questionnaire
8. Height, metabolic weight, and anthropometrics
9. Medication teaching and dispensation
10. Lifestyle and nutrition counseling

Start of Study Drug

At the conclusion of the baseline visit, subjects will be instructed to take the study medication – pitavastatin 4mg PO QD vs. identical placebo – once nightly by mouth. At each subsequent visit, un-used study drug will be collected and a new supply of study drug will be dispensed.

4 Week Visit
1. Interval H&P and side-effect assessment

12 Week Visit
1. Interval H&P and side-effect assessment
2. Fasting blood samples (Fasting glucose, insulin, HbA1c, ALT, AST, CoQ10, pro-insulin)
3. Height, weight, and anthropometrics
4. Lifestyle and nutrition counseling

24 Week Visit – Identical to baseline with added oral glucose tolerance test on additional day.
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METHODS

Study Drug

Pitavastatin (vs. identical placebo) will be administered at a dose of 4mg once daily. Pitavastatin is FDA approved for patients with primary hyperlipidemia or mixed dyslipidemia as an adjunctive therapy to diet to reduce elevated total cholesterol, low-density lipoprotein cholesterol, apolipoprotein B, triglycerides, and to increase high-density lipoprotein cholesterol. Exclusion criteria are designed to exclude subjects for whom pitavastatin use may be contraindicated, including those with unexplained persistent and significant transaminase elevations, and those taking medications with which pitavastatin use is contraindicated or dose-limited. An exemption from IND requirement has been granted by the FDA for this protocol. Management of possible side effects will be as follows:

Myopathy: The risk of myopathy with pitavastatin is low. For patients complaining of muscle aches that are significant and/or persist beyond 1-2 days, a creatine kinase (CK) level will be checked.

- CK > 10 times the upper limit of normal (ULN) will prompt immediate discontinuation from the study.
- If CK is >5 times ULN but <10 times ULN, subjects will discontinue the study drug. If symptoms do not resolve within 2 weeks, they will be discontinued from the study and referred to their provider for evaluation of other possible. If symptoms resolve in <2 weeks, subjects will then restart on pitavastatin 1mg. If symptoms do not return, they will increase to 2mg after 1 week and then to 4mg daily after another week. If symptoms return, they will be discontinued from the study.
- For CK < 5 times ULN, a clinical decision will be made based on severity of the muscle aches. If symptoms are mild, subjects may choose to continue in the study. Alternatively, subjects may be discontinued with restarting of pitavastatin after resolution as above. For myopathy that is judged to be very severe subjects will be discontinued.

Other lab abnormalities: Per guidelines, CK, ALT, and AST will not be sent routinely during the study for patients without symptoms. (ALT and AST at 3 months will be frozen for batched analysis.) If incidental abnormalities in CK, AST, ALT, or creatinine on clinical labs are brought to our attention: (i) CK will be handled as above; (ii) those with Creatinine > ULN confirmed on repeat testing will be discontinued from the study; (iii) those with AST or ALT >3 times ULN confirmed on repeat testing will be discontinued from the study. For elevations below this threshold, response will be based on the degree of elevation, the degree of rise from screen

visit, and any other possible etiologies and concomitant symptoms, and action will be in conjunction with the patient's primary provider.

^{1}H Magnetic Resonance Spectroscopy(MRS) & MRI
After a 12-hour overnight fast subjects will undergo ^{1}H -MRS of the liver and MRI of the abdomen for quantification of liver fat and VAT and SAT, respectively. For liver fat, a breath-hold true fast imaging with steady precession sequence will be obtained. A voxel measuring 20 x 20 x 20 mm (8 ml) will be placed within the right lobe of the liver, avoiding vessels or artifact, and with position within the liver confirmed by 3 planes of view. ^{1}H -MRS data will be acquired using point-resolved spatially localized spectroscopy pulse sequence without water suppression. Proton density fat fraction will be calculated from integral lipid and water peak areas as previously described[30,31]. For abdominal visceral and subcutaneous fat volumes, conventional MR images will be acquired and will serve as anatomic reference of 1H-MRS overlays. Image series will include Axial T1-weighted localizers. No intravenous contrast will be used. The total amount of time in the magnet will be approximately 1 hour.

Subjects undergoing MRI/MRS may develop claustrophobia during the scan. Patients will be queried regarding a history of claustrophobia and their ability to tolerate other MRIs during their screening visit. If they report prior claustrophobia or develop distress during the study MRI, they will be offered a low-dose benzodiazepine (1 mg lorazepam orally) at the time of their scan. Patients who receive anxiolytics will be monitored for a return to baseline mental status following the scan, and will be provided with a ride home.

Whole Body DEXA will be performed to determine total body and regional percent fat and lean body mass. The technique has a precision error (1 SD) of 3% for fat and 1.5% for lean body mass[32]. Trunk, extremity and trunk to extremity ratio will also be assessed[33,34].

Nutritional Analysis
Four day food record at baseline and 6 months will be analyzed for protein, carbohydrate, fat, micronutrient, dietary supplements and alcohol intake (Nutrition Data Systems).

Anthropometric Measurements
Measurements of waist and hip circumference, waist to hip ratio, leg circumference, arm circumference, and neck circumference will be performed using a standardized technique[35].

Activity
Modifiable Activity Questionnaire (MAQ) will be used to assess physical activity[36].

Stable Isotopes and Clamp Subjects will undergo stable isotope and euglycemic hyperinsulinemic clamp studies, in order to assess hepatic insulin sensitivity (stable isotopes and low dose clamp to measure insulin suppression of gluconeogenesis) and peripheral insulin sensitivity ("full" dose clamp). Assessment of hepatic gluconeogenesis will utilize 6,6-2H-glucose. Starting after IV placement, priming dose (3.0 mg/kg) of 6,6-2H-glucose will be administered intravenously, followed by a 4- hour continuous infusion (0.03 mg/kg/min) of 6,6-2H-glucose. Baseline fasting samples for isotopic analysis will be collected at 20, 10, and 0 minutes before the start of the infusion, and samples will be collected at 60, 100, 110, and 120 minutes after the start of the infusion for 6,6-2H-glucose enrichment for assessment of fasting gluconeogenesis. At 2 hours after the start of the isotope infusion, (time = "0"), a "low dose" euglycemic hyperinsulinemic clamp will be performed with an insulin dose of 20 mU/m^2/min to detect differential suppression of hepatic gluconeogenesis. At time=120min, a "full dose" insulin clamp will commence with an insulin infusion of 80mU/m^2/min. Samples for isotopic enrichment

will be assayed by Metabolic Solutions. Peripheral insulin sensitivity will be determined during the last 20 minutes of the full dose clamp using the DeFronzo method[37]. Clamp procedure will be as follows.

- Insulin infusate will be prepared by the MGH pharmacy.
- Two intravenous (IV) catheters will be inserted: (1) one placed in the hand when access is available, otherwise in the forearm or antecubital space, to be used for venous sampling; (2) one placed in the antecubital space or proximal forearm and used for administration of stable isotopes, insulin, and 20% dextrose as below.
- The hand/forearm with the IV that will be used for venous sampling will be placed in a warming box for the duration of the clamp procedure, although it may be withdrawn if the subject finds the warmth uncomfortable.
- At time "0", a priming dose of 100mU/m^2/min of insulin will be given for 2 minutes followed by a continuous infusion of 20mU/m^2/min for the next 118 minutes, comprising the "low dose" clamp.
- At 120 minutes, the dose of insulin will be increased to a priming dose of 400mU/m^2/min for 2 minutes followed by a continuous infusion of 80mU/m^2/min from 122-240 minutes, comprising the "full dose" clamp.
- From 0-240 minutes, a sample (≤ 0.5cc) of venous blood will be drawn every 5 minutes for assessment of glucose using a Hemocue glucose analyzer. Each sample is run twice in the Hemocue analyzer, with an additional third run if the difference between the first two runs is ≥4mg/dL. Samples for serum insulin concentration will be drawn at times 0, 80, 100, 120, 200, 220, 240.
- Starting at 5 minutes, a variable 20% dextrose infusion will be administered to achieve a target glucose of 90mg/dL (range 85-95mg/dL). The rate will be adjusted by the investigator, an MD or NP who is trained in clamp procedure. The initial infusion rate of 20% dextrose will be 0-30cc/hr, with the exact rate determined by the investigator based on the subject's fasting glucose, available clinical information, and, if available, data from the subject's previous clamp procedure for the study. (For example, a subject who is known clinically to be insulin resistant and has a fasting glucose of 110mg/dL would be started at a rate of 0cc/hr, whereas a lean, muscular subject who exercises regularly and has a fasting glucose of 70mg/dL would be started at 30cc/hr.)
- The rate of 20% dextrose infusion will be adjusted up or down for the duration of the clamp procedure by the investigator. Rate changes of 0-40cc/hr will be made by the investigator every 5 minutes, based on the subject's venous glucose and rate of change in glucose, to achieve target glucose of 90mg/dL.
- At 240 minutes, the insulin infusion will be discontinued, and the glucose infusion will continue for 30 more minutes. During this time, subjects will be given a meal. At 270 minutes, venous glucose will be checked, and the glucose infusion will be discontinued if venous glucose ≥80mg/dL. If glucose is <80mg/dL, the glucose infusion will be continued and will be weaned by the investigator at the bedside, with repeat venous glucose sampling every 10 minutes until the glucose is ≥80mg/dL and the infusion is stopped.
- After the clamp, all subjects are counseled concerning the symptoms of hypoglycemia and are asked to report immediately any symptoms. Subjects are observed on the CRC for at least 20 minutes following discontinuation of glucose infusion.

Oral Glucose Tolerance Test A standard 75g Oral Glucose Tolerance Test will be performed with measurement of insulin, c-peptide, and glucose at 0, 30, 60, 90, and 120 minutes.

Lifestyle and Nutritional Counseling will be provided by the CRC bionutritionists at baseline, 12, and 24 weeks based on the 2010 Dietary Guidelines for Americans (US Departments of Agriculture and of Health and Human Services) and the 2008 Physical Activity Guidelines for Americans (US Department of Health and Human Services).

6. Biostatistical Analysis

Data Interpretation: Co-primary endpoints are (i) change in insulin-stimulated glucose uptake during standard euglycemic hyperinsulinemic clamp, reflecting whole-body insulin sensitivity, and (ii) change in hepatic fat fraction as measured by ^{1}H-MRS after 6 months of randomized treatment. Secondary endpoints will include changes in the following: insulin suppression of hepatic gluconeogenesis, fasting glucose, QUICKI, HbA1c, pro-insulin, ALT, AST, and lipids. The analysis will be based on intention to treat population using all available data, including interim data on subjects not completing the study. Wilk-Shapiro test will be used to assess for normality of distribution of all variables, and variables that are not normally distributed will be appropriately transformed. Baseline variables will be compared between treatment groups, and any baseline variable that is statistically different between treatment groups will be adjusted for in subsequent analysis. For variables assessed only at baseline and 6 months, including changes hepatic fat and insulin-stimulated glucose uptake, the effect of pitavastatin vs. placebo will first be assessed first using a two-sample t-test to compare the mean changes. Adjusted analyses will then be performed to control for any baseline differences between treatment groups, potentially including presence of diet-controlled diabetes. We will also perform paired t-testing within the pitavastatin group to assess for any changes in insulin-stimulated glucose uptake.

We will also carefully assess changes in physical activity and dietary intake in terms of overall caloric intake and macronutrient composition between the groups, and we will determine if changes in body weight, physical activity or dietary intake are contributing to the observed treatment effect on insulin-stimulated glucose uptake or hepatic fat. If so, we will include these variables in the analyses and modeling as adjusted covariates. If any other variables are different between groups, such as medication adherence, these will also be included as co-variates. For variables measured at baseline, 3 and 6 months in the study, including AST, ALT, and fasting measures of glycemia, we will analyze the longitudinal data including 0, 3, and 6 month data using general linear mixed effects modeling for which the subject level intercept will be random, and effects of treatment group, time, time x treatment group will be random, and a compound symmetry error covariance structure will be considered. The longitudinal treatment effect between pitavastatin and placebo will be examined by testing for time x treatment group interaction. If there is evidence that any of the outcomes is not normally distributed, we will choose a proper transformation for normalization before the mixed effects model analyses.

Sample Size and Power Calculations:

The sample size of 50, allowing for 10% discontinuation rate, has approximately 80% power to detect a clinically relevant 0.86 SD change in the endpoints of interest at a two-sided alpha of 0.05. Based on preliminary data from study of obese individuals in our group, a 0.86 SD change in insulin-stimulated glucose uptake is a change of approximately 1.5mg/kg/min. A difference of less than this magnitude in change over time compared to placebo would not be viewed as clinically relevant, and consistent with the hypothesis that pitavasatin does not significantly affect insulin resistance. Moreover a difference of greater than 0.86 SD in change in hepatic fat between the groups would be viewed as clinically relevant, and consistent with the hypothesis that pitavastatin will reduce liver fat. Power for within group paired t-testing to assess difference in insulin stimulated glucose uptake within the pitavastatin group will depend on the correlation between baseline and 6 month measurements, which we estimate will be between r = 0.6 to 0.8. Estimated power for N = 20 to 23 pairs is shown below:

r (correlation between baseline & 6 month measurements)	Detectable difference in means	N (subjects with both baseline & 6 month)	Power to detect difference in means
0.6	≥ 0.6 SD	20	0.812
0.6	≥ 0.6 SD	21	0.832
0.6	≥ 0.6 SD	22	0.851
0.6	≥ 0.6 SD	23	0.867
0.7	≥ 0.5 SD	20	0.782
0.7	≥ 0.5 SD	21	0.803
0.7	≥ 0.5 SD	22	0.823
0.7	≥ 0.5 SD	23	0.841
0.8	≥ 0.5 SD	20	0.918
0.8	≥ 0.5 SD	21	0.931
0.8	≥ 0.5 SD	22	0.942
0.8	≥ 0.5 SD	23	0.952

7. Risks and Discomforts

Radiation: As a result of participation in this study, subjects will be exposed to 2 DXA scans over 6 months, for a total radiation risk for both scans of 0.02mSv. This radiation exposure does not pose excessive risk to subjects.

Blood Drawing: The total blood drawn for subjects completing the study is equivalent to approximately 510cc over a period of 6 months. The maximum amount of blood drawn at any given visit will be approximately 200cc, which will be drawn at the baseline and 6 month visits. A total of 510cc (a little more than a blood donation) over 6 months does not pose excessive risk to patients, nor does withdrawal of up to 200cc at a single timepoint. Both of these amounts are within Institutional Review Board guidelines. Patients with a hemoglobin < 10 g/dL will be excluded from the study. There will be minimal risk and discomfort associated with blood drawing and IV placement. The risks of these procedures are minor bruising or bleeding at the site of the blood draw or IV catheter.

Euglycemic Hyperinsulinemic clamp/insulin: Administration of insulin infusion can cause hypoglycemia. Blood sugar will be monitored every 5 minutes during the insulin clamp, and 20% dextrose is infused per protocol to achieve target blood glucose of 90mg/dL. In the event of hypoglycemia, 50% dextrose is available at the bedside, and a physician or nurse practitioner is present throughout the insulin clamp procedure.

Medications
Administration of all medications will be conducted by the appropriate health care professional. A physician will be available on-call 24 hours/day, 7 days/week, to all study participants for any questions or concerns. Subjects will be followed closely for symptoms and will be instructed to call the study physician with any concerns. Please see “Study Drug” section above for handling of symptoms and adverse events.

Subjects who enter the study may meet criteria for statin treatment based on new guidelines. Only subjects who are not currently receiving a statin and who are not under consideration to be

prescribed a statin will be entered into the study. Half of patients will be randomized to placebo for six months, after which they will be referred to their provider. A potential delay of 6 months in initiation of statin for patients not currently being considered for statin does not pose excessive risk to subjects. All subjects will be informed of their ASCVD risk calculation after screening results are available, and all subjects will be informed that they can seek immediate care from their provider for statin therapy rather than entering the study if they wish.

8. Potential Benefits

Subjects on placebo may not benefit from being in the study. They will receive detailed medical information and will be referred to their physicians at the end of the study for continued care and initiation of statin therapy if appropriate. Subjects on pitavastatin will receive lipid lowering therapy that will potentially be of benefit with respect to primary prevention of cardiovascular disease, given that subjects will have LDL ≥ 100mg/dL or ASCVD risk of ≥5% over the next 10 years.

The study is likely to benefit the overall population of individuals needing statin therapy, as it will elucidate effects of pitavastatin, on glucose homeostasis and liver fat.

9. Monitoring and Quality Assurance

The study investigators will monitor all data collected for the studies. Data will be stored securely, with access restricted to co-investigators and study staff. Binders with subject information will be labeled with coded enrollment number to protect confidentiality. Electronic databases will be locked, and password-protected, with access available only to study staff. Data will not be saved on the hard drive of any laptop or desktop computers or on any removable data storage devices such as flash drives or CDs.

Craigslist and Facebook will also be utilized, with a link to a REDCap Survey that individuals can fill out. This survey will collect minimal information necessary to see if subjects would potentially be eligible, as well as contact information so that we can call them if they are potentially eligible

The PIs (Steven Grinspoon, MD, and Takara Stanley, MD) will monitor the study continuously, reviewing all unexpected and possibly related adverse events as they occur, and they will meet quarterly to review study progress and ensure quality.

Subjects will be instructed to report immediately any adverse events. A physician or nurse practitioner will be available on-call 24 hours/day, 7 days/week, to all study participants for any questions or concerns.

Adverse events will be reported to the MGH Institutional Review Board in accordance with AE reporting guidelines.

10. References

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