Official title: 7 Tesla MRS to Study Statin-Related Muscle Complaints

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Protocol

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1. Introduction and Purpose: Roughly 5-10% of statin-treated patients report muscle pain, aches, weakness, cramps, stiffness, or "heaviness" - typically occurring symmetrically in the legs[1]. For healthcare providers, the major diagnostic challenge is to unambiguously link these symptoms to statin use, especially since some patients can have normal serum creatine kinase (CK) levels despite demonstrable weakness and muscle biopsy proven statin-induced myopathy [2].

The 2103 American College of Cardiology and American Heart Association (ACC/AHA) Blood Cholesterol Guidelines committee advocates statin withdrawal and rechallenge as a way to establish a causal relationship between muscle pain symptoms and statin therapy [3]. This challenge involves stopping the statin for 2 weeks to see if the symptoms go away, then restarting the statin to see if the symptoms return. As long as patients are not under conditions of severe acute vascular stress (e.g. acute coronary syndrome, ischemic stroke, or major vascular surgery), short-term withholding of statin therapy is generally regarded as safe [4].

Several issues limit widespread use of statin withdrawal and challenge as recommended by the ACC/AHA committee. First, health-care providers lack an evidence-based standardized protocol, making the process more of an art rather than a science. Second, the sensitivity and specificity remains unknown. In at least 2 studies, placebo-treated patients develop muscle complaints during controlled withdrawals and rechallenges [1, 5]. Third, the outcome, self-reported muscle issues, remains subjective. No biomarker or imaging technique exists.

No well accepted, standardized, or Food and Drug Administration (FDA)-endorsed diagnostic method exists for statin-induced muscle injury. This lack of an objective diagnostic methodology blocks vertical advancement of the field. For example, clinical investigations cannot enroll patients without using subjective inclusion criteria. In the clinical setting, health care providers have little insight into whether to try switching statins – and thus risking further muscle injury – or switch to non-statin lipid lowering drugs. Payers hesitate to approve new expensive non-statin lipid lowering drugs (e.g. PCSK9 inhibitors) based on subjective complaints of non-specific muscle symptoms. Studies of mitochondrial dysfunction, thought to be related to the mechanism of statin-induced muscle injury, have been limited to preexisting cell lines [6], animal models, or *ex-vivo* human experiments with small sample sizes [6, 7] (mostly because such studies require invasive muscle biopsies).

UT Southwestern has one of the few available 7 Tesla (7T) magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) instruments. The 7T MRS can detect ATP synthesis rates in human skeletal muscle as well as study intramyocellular lipids, carnitine and acetylcarnitine in the trimethylamine signals after exercise, lactate dynamics, and phosphorus metabolite kinetic exchanges. Compared to 3 Tesla and 1.5 Tesla, 7T offers much higher detection sensitivity and increased spectral resolution. Patients' scan times are short and the data quality better. 7T also helps identify small metabolite changes that otherwise are difficult to observe at lower fields.

The central hypothesis of this proposal is that statin-induced myopathy causes changes in muscle metabolism that can be detected by a 7 Tesla MRS.

The successful completion of this project will develop in vivo techniques that will provide insight into how statins affect muscle metabolism and help establish a methodology to objectively diagnose muscle injury due to statins. The development of an MRS technique will allow for *in-vivo* analyses and the data accumulated here will serve as preliminary data for futher extramural funding of studies with

much larger sample sizes. Ultimately, this focus of research will lead to improved diagnosis and treatment of patients with statin-related muscle complaints, which is central to obtaining the cardiovascular risk reduction from lipid-lowering drugs.

2. Background: The first case of statin-induced muscle injury occurred soon after the discovery of statins by Drs. Akira Endo and Masao Kuroda in Japan. They administered high doses of mevastatin to a 17-year-old girl with homozygous familial hypercholesterolemia [8] and noted "a side effect (muscular weakness at the proximal parts of the extremities with a rise of serum creatine phosphokinase, glutamate-pyruvate transaminase and glutamate-oxaloacetate transaminase activity)." They also noted "these side effects completely disappeared within two weeks after withdrawal of the drug." With lower doses of the mevastatin, "the side effects were no longer experienced." In 1988, the *New England Journal of Medicine* published the first cases of rhabdomyolysis with statins: in two separate reports (one from UT Southwestern Medical Center), five cardiac transplant patients were described as developing rhabdomyolysis after taking lovastatin [9, 10].

Despite awareness of statin-induced muscle injury, the exact pathology at the level of skeletal muscle remains unclear. Several contributing factors have been investigated without clear evidence of causality.

Muscle biopsy studies have varied findings, although most recent investigations suggest decreased mitochondrial function. It remains unclear why mitochondrial content decreases. Most published studies imply decreased Coenzyme Q10, an intermediate in the cholesterol synthesis pathway that is decreased with statin use. Yet interventional studies with CoQ10 supplements have yielded conflicting results for improving statin-related muscle complaints [11, 12]. Within mitochondria, changes in complex 1 and complex III have imply altered oxidative phosphorylation [7].

Genetic studies have linked statin-induced myopathy to genes involved in drug metabolism without providing much insight into the pathology at the level of the muscle. The best studied of these genes is solute carrier organic anion transporter family member 1B1 (*SLCO1B1),* which encodes for OATP1B1. A recent genome wide association study identified an association of simvastatin-related muscle injury and the nonsynonymous rs4149056 single-nucleotide polymorphism (SNP) in *SLCO1B1* [13] in patients enrolled in a clinical trial of simvastatin 40 mg vs simvastatin 80 mg daily. More than 60% of myopathy patients had the minor C allele (odds ratio 4.3 for one C allele and 17.4 for both C alleles). However, our preliminary data (see below) ascertained from clinic patients (rather than patients enrolled in clinical trials) failed to identify this SNP as a risk factor for statin-related muscle complaints.

A small subset of patients with statin-related muscle complaints develop antibodies to 3-hydroxy-3-methlyglutaryl coenzyme A reductase (HMGCR)[14]. The estimated incidence of anti-HMGCR-associated autoimmune myopathy is 2 per million per year. Such patients have muscle weakness that continues even after statins are withheld and responds to treatment with immunosuppressive therapy. Such patients should not be rechallenged with statins.

Three prior studies utilizing MRS have been published [15-17], none in the past 5 years and none with a 7 T MR system. Results have been variable - likely related to small sample sizes and differing inclusion criteria – but have established that statins induce muscle changes detectable by MRS techniques.

Most recently, Wu et al studied 3T MRS findings in the posterior calf of 10 subjects (no control group was included and only 4 of whom had any history of statin induced muscle complaints) before and after 4 weeks of statin therapy [17]. They observed an increase in metabolic recover time from 28.1 seconds to 55.4 seconds ($p = 0.02$) as measured by changes in phosphocreatine peaks before, during, and after exercise. These findings imply impairment in mitochondrial oxidative metabolism. An

older study, done on forearm flexor muscles with a 4.7 T MRS, found no difference in phosphocreatine peaks in 7 patients with a history of statin-induced rhabdomyolysis (not on statins at the time of the study) compared to 7 control patients on statins without any muscle complaints [16]. Rather, the study found a slower rate of pH recovery (10.3 vs 15.7 mM H+/minute, $p < 0.05$), which the authors related to possible statin-induced defects in ion transport. Finally, Slade et al studied 3T MRS findings in the anterior tibialis muscle of 10 statin users (without any history of statin related muscle complaints) compared to 10 controls and found that the phosphodiester peaks were 57% higher in statin users, implying increased cell membrane turnover similar to muscular dystrophies [15].

Preliminary Data: As part of an ongoing study of a familial hypercholesterolemia (FH) cohort, we studied statin-related muscle complaints in 278 FH patients recruited from lipid clinics in the Dallas, TX area (manuscript in submission). Statin-induced myopathy occurred in 36% (n = 97). One patient had a reported history of statin-induced rhabdomyolysis and 2 patients had elevations in CK that were clearly linked to statin use. Simvastatin was the most common statin linked to muscle complaints, and the risk of myopathy was associated with age (OR 1.6, [95% CI 1.2, 2.2]), BMI in non-African-Americans (0.90 [0.83, 0.97]), and hypertension (0.4, [0.2, 0.9]). In patients who were unable to reestablish statin therapy, LDL-C levels remained markedly elevated ("eventually tolerant" 127 vs."never tolerant" 192 mg/dL, $p < 0.001$).

SLCO1B1 rs4149056 genotyping revealed 224 wild-type patients (TT), 48 heterozygotes (TC), and one homozygote (CC). The variant C allele was not associated with the risk of statin-induced myopathy (OR 0.70, [95% CI 0.37, 1.33]).

3. Concise Summary of Project:

We plan to do 2 substudies, running concurrently, in this proposal. In each one, visits will be for approximately 3.5 hours. A written informed consent will be taken from all subjects. All visits will be to the Advanced Imaging Research Center at UT Southwestern for study procedures (blood testing and MRI/MRS). Urine pregnancy test will be collected in women of childbearing potential. Muscle pain will be evaluated with a pain assessment scale. All patients will be counseled to not alter exercise habits or diets for the duration of the study.

The goal of the first study will be to generate hypothesis regarding the long-term effects of statin in muscles by studying 7T MRS findings in patients who are currently experiencing statin-related muscle complaints or who have a history of severe reactions to statins (i.e. rhabdomyolysis, anti-HMGCR-associated autoimmune myopathy, or CK elevation > 10 times the upper limit of normal). We anticipate enrolling roughly 5 such patients. The protocol involves a single visit for a blood draw and MRS of calf muscle. The data will be compared to historical controls provided by the Advanced Imaging Research Center.

Table 1: Substudy 1 Procedures

	Visit 1
7T MRS	X
Blood Draw	X
Pain Assessment	X

Second, We plan to study the effect of short-term statin administration in 5 patients with a history of statin related muscle complaints (currently do not suffer related muscle pain) and 5 controls with no statin related muscle complaints (Table 2). Controls will be matched for age, weight, and body mass index. Patients will undergo a wash-out of lipid lowering drugs followed by a challenge with simvastatin 40 mg daily (similar to a statin withdrawal and re challenge). We chose simvastatin since it is the most common statin that caused myopathy according to our preliminary data (as well as most other published reports).

Each patient will have 5 visits.

The screening visit will involve a review of inclusion/exclusion criteria, blood draw, questionnaire, and instructions to withhold all lipid lowering drugs until visit 1. Any patient with known antibodies to HMGCR will be excluded from the remainder of the study.
Visit 1 will occur 2 weeks after stopping all lipid lowering drugs. A minimum 2 week period off lipid lowering drugs is required to allow clearance of any medication from the systemic circulation. Patients will undergo MRS of the calf muscle and a blood draw. Patients will then start simvastatin 40 mg daily.
Visit 2 will occur 1 week after starting simvastatin and will get MRS and blood draw.
Visit 3 will occur 2 weeks after starting simvastatin and will get MRS and blood draw.
Visit 4 will occur 4 weeks after starting simvastatin and will get MRS and blood draw.

Even though most patients note the reappearance of muscle symptoms within the first week of the rechallenge period, we have extended the study to 4 weeks. During this 4 week time period we will capture all the metabolic and chemical changes and will be able to correlate better with the statin induced myopathy. These serial MRS scans will allow us to collect data on when abnormal MRS signals start to appear. This data will serve as pilot data that will allow us to better design future studies.

For safety purposes, if patients develop muscle symptoms during the simvastatin rechallenge, they will come for a study visit immediately so that an MRS can be done. Then, they will stop the simvastatin.

Table 2: Sub Study 2	Procedures				
	Screening	Visit 1	Visit 2	Visit 3	Visit 4
7TMRS		X	X	X	X
Blood Draw	X	X	X	X	X
Pain Assessment	X	X	X	X	X
Start Simvastatin 40 mg daily		X	X	X	X

4. Study Procedures:

The following procedures will be performed:

Demographic Characteristics, Health History, and Physical Examination
Subjects will be asked to complete a questionnaire on demographic characteristics and will also be asked questions about their medical and medication history, as well as, general physical activity level. The subject will also have their height, weight and vital signs measured.

Blood Draw
The following will be tested:

- Serum: chemistry, renal, liver, & thyroid function tests, lipid panel, lactate, CK, HMGCR antibodies
- Whole blood: CBC w/ differential, estimated sedimentation rate, Hemoglobin A1C
- Urine: pregnancy tests in females if indicated.
- Genetic Testing: Two aliquots will be used for DNA extraction.

Magnetic Resonance Spectroscopy (MRS) of Calf Muscle
Subjects enrolled in the study will undergo magnetic resonance spectroscopy (MRS) of calf muscles in a high field magnet. The Advanced Imaging Research Center at UT Southwestern now has 7T

systems for human experiments. While conventional MR techniques will be used, some MR parameters will vary. Compared to 3T and 1.5T, 7T offers much higher detection sensitivity and increased spectral resolution. Patients' scan times are short and the data quality better. Seven Tesla also helps identify small metabolite changes that otherwise are difficult to observe at lower fields. All tests performed will be within the recommended operating conditions deemed to be a non-significant risk by the Food and Drug Administration (FDA).

The subject will be screened for metal in or on their body using a standard MR screening form. Any female of childbearing age will be screened for potential pregnancy. The subject will then remove all metal from their body and change into a gown or scrubs prior to admittance to the 7T MR environment. Some metabolites are better detected after exercise. Some volunteers who do/do not regularly exercise or are/are not active in sports may be asked to exercise prior to the MRS. This exercise may be in the form of hand grasps, toe-raises, bicycling on a stationary bike or walking on a treadmill. Exercise will be limited to up to one hour, during which time the subject's vital signs and blood oxygenation will be monitored. During the scan, the subject's heart rate and respiratory rate may be monitored. Calibration and set up of the scanner takes approximately 20 minutes. This is done when the subject is in the scanner; therefore, the time in the scanner for the subject will be approximately a total of 2 hours, after which time the subject will be free to leave.

5. Sub-Study Procedures: Not applicable.

6. Criteria for Inclusion of Subjects:

Inclusion criteria for substudy 1 include:

1.) Adults, age 18 years or older
2.) One of the following:
    a. Patients who are currently taking a statin and experiencing muscle pain, aches, weakness, cramps, stiffness, or "heaviness" in the legs. Since the MRI/MRS studies will be limited to calves, patient will have to have leg symptoms to qualify, or
    b. Patients with HMGCR antibodies, or
    c. Patients with a history of statin-induced CK elevation of > 10 times the upper limit of normal, or
    d. Patients with a history of statin-induced rhabdomyolysis
3.) Subjects should be willing to participate under the conditions described in the informed consent form (ICF) and must be able to sign the ICF and applicable HIPAA forms.
4.) Ability to exercise without physical restrictions.

Inclusion criteria for substudy 2 include:

1.) Adults, age 18 years or older
2.) Patients who report complaints of statin-related muscle pain, aches, weakness, cramps, stiffness, or "heaviness" in the legs. Since the MRI/MRS studies will be limited to calves, patient will have to have leg symptoms to qualify.
3.) Subjects should be willing to participate under the conditions described in the informed consent form (ICF) and must be able to sign the ICF and applicable HIPAA forms.
4.) Ability to exercise without physical restrictions.

7. Criteria for Exclusion of Subjects:

Exclusion criteria for substudy 1 include:

1.) Any patients with underlying non-statin related muscle disorders such as inflammatory myopathy or myotonic dystrophy.
2.) Presence of any clinically significant uncontrolled endocrine disease known to influence serum lipids or lipoproteins. Note: patients on thyroid replacement therapy can be included if the dosage of thyroxine has been stable for 6 weeks prior to the first study visit and thyroid-stimulating hormone (TSH) level is within the normal range.
3.) Conditions of severe acute vascular stress (e.g. acute coronary syndrome, ischemic stroke, or major vascular surgery) within 3 months prior to the first study visit.
4.) History of rheumatological disease associated with symptoms that may be confounded with statin-related muscle complaints, e.g. rheumatoid arthritis
5.) Diagnosis of fibromyalgia
6.) Pregnant or breast-feeding women. The effects of high magnetic fields on a fetus are unknown.
7.) Any person with implanted metal, because of MR safety. This will include exclusion for vascular clips, surgical clips, prosthetic valves, pacemakers, otologic implants, etc.
8.) Use of any active investigational drugs within 1 month or 5 half-lives, whichever is longer.

Exclusion criteria for substudy 2 include:

1.) Patients on the following drugs for which the FDA has issues restrictions for using simvastatin 40 mg daily do to an increased risk of severe muscle injury: itraconazole, posaconazole, ketoconazole, erythromycin, clarithromycin, telithromycin, HIV protease inhibitors, nefazodone, gemfibrozil, cyclosporine, danazol, amiodarone, amlodipine, ranolazine, and verapamil.
2.) Patient who drink large quantities of grapefruit juice (> 1 quart daily)
3.) Patients with muscle-related pain that is not related to statin-use (e.g. muscle aches from strain or trauma) or remains unexplained.
4.) Any patients with underlying non-statin related muscle disorders such as inflammatory myopathy or myotonic dystrophy.
5.) Presence of any clinically significant uncontrolled endocrine disease known to influence serum lipids or lipoproteins. Note: patients on thyroid replacement therapy can be included if the dosage of thyroxine has been stable for 6 weeks prior to the first study visit and thyroid-stimulating hormone (TSH) level is within the normal range.
6.) Conditions of severe acute vascular stress (e.g. acute coronary syndrome, ischemic stroke, or major vascular surgery) within 3 months prior to the first study visit.
7.) Any patients with a history of severe or life-threatening reactions to statins including rhabdomyolysis (defined as evidence of organ damage with CK >10,000 IU/L) , CK elevation > 10 times the upper limit of normal, cognitive decline, or allergic reactions to statins.
8.) History of rheumatological disease associated with symptoms that may be confounded with statin-related muscle complaints, e.g. rheumatoid arthritis
9.) Diagnosis of fibromyalgia
10.) Patients unable to maintain their current activity level or planning to increase their activity level (e.g. new exercise regimen). Such changes may have acute effects on muscle metabolism.
11.) Pregnant or breast-feeding women. The effects of high magnetic fields on a fetus are unknown.
12.) Any person with implanted metal, because of MR safety. This will include exclusion for vascular clips, surgical clips, prosthetic valves, pacemakers, otologic implants, etc.
13.) Use of any active investigational drugs within 1 month or 5 half-lives, whichever is longer.
14.) Antibodies to HGMCR detected in the blood drawn from the screening visit.

8. Sources of Research Material:

Research material will consist of demographic questionnaires and medical history, blood and urine specimens, physical examination, and MRS results.

9. Recruitment Methods and Consenting Process:

The study subjects will be recruited from the lipid clinics at Parkland Health and Hospital Systems and UT Southwestern Medical Center. We will also advertise the study among local physicians. We will post the study on our website and at clinicaltrials.gov.

Interested subjects will contact the Research Coordinator for more information regarding the study. The Research Coordinator will screen the potential subject according to Inclusion/Exclusion Criteria listed above. Both genders as well as minorities will be actively recruited for participation in this study. An approved HIPAA Waiver of Authorization will allow subjects to contact the research coordinator or other investigators for more information regarding study activities and eligibility screening. Subjects will participate after the purpose and benefits of the study are explained by physicians or nurses (i.e., Research Coordinator) associated with the study and signatures on applicable consent forms are obtained. The informed consent process will include a review of a detailed list of potential adverse reactions. The informed consent process will be documented in the subject's research chart. All subjects will receive a copy of their signed ICF and HIPAA forms. Subjects will be paid $100 for each study visit that involves MRS. $100 for Substudy 1 and $400 for Substudy 2.

10. Potential Risks:

Loss of Confidentially: Potential risks include the possible violation of the patient's privacy, since medical history will be used as a source of data.

Genetic testing: DNA will be collected and stored for potential future studies. Genetic testing carries the risk of revealing family links, or information about disease risks, which if revealed, could impact employment or insurance. This risk is minimized by the use of a separate genetic consent which is not kept with the ordinarily accessed hospital chart.

Risks of Blood Drawing: Phlebotomy carries the rare to occasional minimal risk of discomfort, hematoma, infection, fainting and/or vasovagal response.

Withdrawing Lipid Lowering Therapy: Patients will stop all lipid lowering therapy for several weeks, resulting in an increase in cholesterol. As long as patients are not under conditions of severe acute vascular stress (e.g. acute coronary syndrome, ischemic stroke, or major vascular surgery), short-term withholding of lipid lowering therapy is generally regarded as safe. Patients will still get long-term benefits of cholesterol lowering (i.e. reduction and risk of atherosclerotic cardiovascular disease).

Statin-induced Muscle Injury: In substudy 2, patients will be administered a statin, increasing their risk of muscle injury. Such statin challenges, however, are common in clinical practice, and in several published clinical trials, seem to be overall safe. We have taken several measure to minimize this risk: excluding patients with a history of severe muscle injury from statins (e.g. rhabdomyolysis or CK elevations > 10 times the upper limit of normal); excluding patients on drugs that interact with statins and increase the risk of muscle injury; excluding any patients with antibodies to HMGCR; and minimizing the time of the statin exposure.

Magnetic Field: This procedure is experimental and the long-term risks of the high magnetic field are unknown. Exposure to high magnetic fields may have effects on the normal electrical activity of the body including changes in nerve and heart function. Changes in the heart rate or rhythm could occur. Blood pressure, temperature, and pulse rate may change. Studies with an 8 Tesla MRI have not shown any significant effects.

A subject may feel dizzy when moving the head quickly while in the magnetic field. This is a relatively frequent sensation, perhaps occurring in 30% or more of subjects. In studies at 8 Tesla, 1 subject out of 200 has vomited as the result of being in the magnet. A relatively common sensation is a metallic taste in the mouth while entering or exiting the scanner. A subject might see tiny light flashes similar to those that occur when you rub your eyes in the dark. This is less common and has no known harmful effects. Claustrophobia (uneasiness at being in a confined space) is a common contraindication in any MRI procedure at any magnetic field strength (tesla).

Exposure to the rapidly changing magnetic fields could cause twitching of the subject's muscles. This effect, known as peripheral nerve stimulation (PNS), is temporary.

It is possible that radio frequency energy in a 7 Tesla MRI system may be absorbed in an unexpected manner by human subjects. Local heating of the body may occur.

As with all MRI, any metal in the body or in clothing represents a risk. Metallic objects can be accelerated by the magnetic field and become dangerous. A metal implant or foreign object in the body could be displaced by the magnetic field. There can be increased heating near metal structures.

Females: Risks to pregnant women and their fetuses are unknown at high magnetic field. Females who are pregnant or breast-feeding cannot be a part of this study. Females of childbearing age must agree to be tested for pregnancy prior to the MRI study.

11. Subject Safety and Data Monitoring:

All potential adverse events will be discussed with study participants. Subjects will be informed of the voluntary nature of this research and will be in constant contact with the instrument operator should they desire to terminate the study for any reason. To minimize dizziness, subjects will be moved slowly in and out of the magnet. Earplugs and earmuffs will be provided to minimize discomfort of acoustic noise from the scanner sequences. If a subject experiences muscle twitching, they will be asked to inform the operator and the study will be discontinued. Additionally, if a subject states they feel an area of their body getting warm, they will be asked to notify the operator and the scan will be stopped. All subjects will be carefully evaluated prior to entering the magnet to exclude metal objects in or on their body. A person with metal implants will be excluded from participation in the 7T scanner. Females of childbearing age will have a pregnancy test prior to any MR procedure. All subjects will complete a 24 hour post scan evaluation form.

In the event of a medical emergency, the Aston Ambulatory Emergency Response Team, which consists of qualified medical personnel physically located in the UTSW north campus, may be contacted during ordinary work hours for emergency assistance.

12. Procedures to Maintain Confidentiality:

Confidentially will be maintained per HIPPA guidelines. Any information obtained in connection with this research that can be identified with a subject will remain confidential and will be disclosed with the permission of the subject or subject's legal guardian. All individuals who have access to data obtained during this study will have approved human subjects protection training and will provide written assurance of compliance with the statements in the Belmont Report, 45 CFR 46, and Multiple Project Assurance M- 1304. The IRB may review research information if needed for auditing or other purposes that the IRB deems necessary. Data bases, research files and samples will include ID numbers only. All aliquots or derivatives of initial samples will be labeled with ID numbers only; no personal health information (PHI) will be included. The ID numbers will not be derived from PHI. Obtained data and subject identification information will be kept separate. All medical and biological information will be confidential and no disclosures of personal identity will be allowed unless specifically requested by the research subject or subject's legal representative. The location of copies

of executed consent forms will be kept in a locked research office. The consent will be kept securely and separately from the subject's medical chart.

13. Potential Benefits:

Knowledge gained by this project may lead to insight into how statins affect muscle metabolism as well as develop an objective methodology to diagnose statin-induced muscle injury. Objective methodology would allow for not just definitive diagnoses in the clinical setting, but also would provide inclusion criteria for clinical trials or further studies of statin-related muscle injury. Understanding of statin induced muscle injury could help establish new therapies and strategies in prevention of statin related muscle injury. Ultimately, better understanding of statin-related muscle complaints would help tailor therapy and improve adherence, critical to reducing the burden of atherosclerotic cardiovascular disease.

14. Biostatistics: Unpaired nonparametric tests will be used to compare data from both groups in substudy 2. $P < 0.05$ will be the significant threshold.

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