Official title: Statin-associated muscle symptoms: in vivo and in vitro studies of mitochondrial function

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1. Project Description

1.a *Overview*. Over 40 million Americans take statins to reduce their risk of atherosclerotic cardiovascular disease (ASCVD). Unfortunately, 10 to 20% stop taking them due to statin-associated muscle symptoms (e.g. pain, aches, weakness, cramps, or stiffness) (1, 2). The pathophysiology of these statin-associated muscle symptoms (SAMS) has remained elusive. Consequently, no objective diagnostic method exists, causing confusion for patient and providers since muscle symptoms can often be multifactorial.

The *overall objectives* of this project are to identify the underlying cause of SAMS and establish an *in-vivo* imaging technique to detect SAMS. **The *central hypothesis* of this proposal is that statins directly inhibit mitochondrial function in SAMS patients.** Our rationale is based on our own preliminary data indicating that simvastatin – the most common statin to cause SAMS – can directly inhibit oxidative phosphorylation (OXPHOS) in mice. Since such changes can be detected *in vivo* in humans utilizing ^{31}P magnetic resonance spectroscopy (MRS) techniques, we will use a state-of-the art 7 Tesla (7T) MRS instrument to study the soleus muscles of SAMS patients. Additionally, we will validate the MRS findings by doing functional studies in muscle biopsy specimens.

We propose double-blind randomized, placebo-controlled pilot study in 15 SAMS patients and 15 controls. Study participants will be treated with simvastatin 40 mg daily or placebo for 10 weeks. We will perform 7T MRS of soleus muscles at randomization and either at first complaint of muscle symptoms or at the end of 10 weeks if no muscle symptoms occur, whichever occurs first. Quadriceps muscle biopsies will also be done immediately following the second MRS scan.

1.b *What is the scientific problem that will be addressed and why is this important?*

SAMS are prevalent and limit the benefits of lipid lowering. Statins are the second most commonly prescribed medication class in the US (3). Unfortunately, muscle injury – ranging from mild muscle aches to life-threatening rhabdomyolysis – limits their use. Based on observational data, 10-20% of statin users stop treatment within the first year of the initial prescription, citing SAMS as the primary reason (1, 2). Even among adherent statin users, one-third experience interruptions in therapy due to SAMS (4).

The current paradigm for treating SAMS patients is to diagnose subjectively and use trial-and-error approaches to reestablish therapy. These reestablished statin therapy are often at lower doses or with less potent statins, resulting in under-treatment (5-7). Also, since new lipid lowering agents (e.g. PCSK9 inhibitors) are expensive, payers often are unclear as to whether to cover the cost for patients with subjective complaints.

The major gap in knowledge is the effect of statins on muscles. The pathophysiology of SAMS remains unclear. Human genetic studies have implicated genes in hepatocyte drug transport pathways (8), but have failed to provide insight into what is occurring in muscle tissue. Translational studied have raised several possibilities (e.g the roles of Coenzyme Q10, Vitamin D, and circulating cholesterol levels), but results of clinical trials and human genetic studies have failed to support any of these (9-15).

Statins may have an off-target effect: inhibition of mitochondrial function. Our preliminary data indicate that simvastatin lactone (the form that is highly cell permeable)

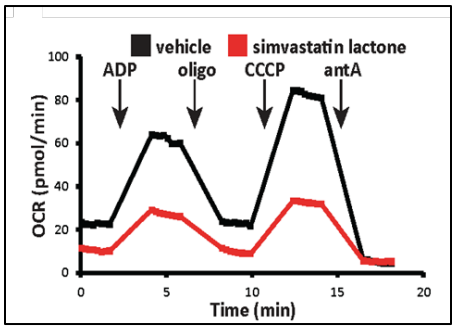


Figure 1: Oxygen consumption rates (OCRs) from mitochondria (0.1mg) isolated from wild type mouse skeletal muscle (tibialis anterior) treated with vehicle or simvastatin (lactone, 100uM) in the presence of complex I substrates (glutamate/malate). Drugs (ADP, oligomycin (oligo), CCCP, antimycin A(antA)) were injected at times indicated.

reduces oxygen consumption rates in isolated mitochondria (Figure 1). Our findings are consistent with prior *in silico* data identifying a binding site for simvastatin in mitochondrial complex III (16).

1.c *What are the innovative or high-risk, high-reward approaches that, if successful, might lead to paradigm-shifting results?*

We will be able to correlate 7T MRS findings to studies of mitochondrial structure, function, and gene expression. The studies proposed here will allow for an integrated understanding of *in vitro* and *in vivo* changes induced by statins. If our hypothesis is correct, this work will lead to ***objective markers*** (e.g. MRS signals) that can be used to detect SAMS. Such a tool would shift the current paradigm (i.e. subjective diagnosis) by providing a tool to help providers, patients, and payers determine whether a statin is truly the cause of muscle symptoms. Such a tool could also be used to identify appropriate patients for further studies and follow their therapeutic response.

1.d *Does the project address a critical barrier to progress in the field?*

Human studies are required since no adequate animal model exists. SAMS has been difficult to induce in preclinical models with statins other than cerivastatin (17-20), perhaps because of lack of muscle exposure when statins are given systemically to animals.

No standardized diagnostic criteria exist for SAMS. The status quo as it pertains to SAMS is to diagnose patients based on their subjective complaints, resulting in confusion for patients, providers, payers, and clinical investigators. Statin withdrawal and rechallenge is often advocated as a diagnostic tool (21), but no evidence-based standardized protocol exists, the sensitivity and specificity remains unknown, and the outcome (self-reported muscle symptoms) remains subjective. A few commercially available diagnostic tests may be used for SAMS patients: blood creatine kinase (CK) levels, solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) rs4149056 genotype, and antibodies to 3-hydroxy-3-methlyglutaryl coenzyme A reductase (HMGCR). However, only 5% of SAMS patients develop elevated CK levels, SL*CO1B1* rs4149056 genotype only predicts the risk of extreme elevations in CK in patients on simvastatin (8, 22, 23), and antibodies to HMGCR are responsible only for cases with severe CK elevations (greater than 1000 U/L) that fail to resolve after statins are withdrawn (24). *Thus for 95% of SAMS patients, no diagnostic test helps diagnose SAMS.*

The proposed research addresses this critical barrier to progress in the field by utilizing a 7T MRS instrument to develop an *in vivo* technique that will objectively diagnose SAMS, effectively removing a major roadblock for the 4-8 million existing SAMS patients. Our findings have the promise of improving the care of patients with SAMS, allowing them to benefit from life-long lipid lowering therapy and reduced cardiovascular risk.

1.e *Are novel approaches, methodologies, tools or technologies being used?*

We will use a state-of-the art 7 Tesla MRS Instrument to study the effects of statins on mitochondrial function. Three prior studies utilizing MRS to study the effects of statins have been published (25-27). None were done in the past five years, and none with a 7T field. Their results were varied, likely due to limitations with lower field MRS instruments such as reproducibility and long scan times (28).

The UT Southwestern Advanced Imaging Research Center (AIRC) has one of the few available 7T MRS instruments. Compared to 1.5T and 3T, 7T offers much higher detection sensitivity and increased spectral resolution (29-34). 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.

Our preliminary data indicates that the 7T MRS technique can measure mitochondrial function in SAMS patients. We have performed both resting state and exercise measurements of mitochondrial function in a few SAMS patients. First, we did resting state measurements in 3 SAMS patients and one control patient before and after simvastatin 40 mg daily for 1 week. MRS data was captured using a recently developed method to assess ATP synthesis rates at rest (35-37). The results were varied. Next, we did pre-and post-exercise (i.e. toe lifts for 2 minutes) measurements in 1 SAMS patients before and after starting simvastatin 40 mg daily. She developed muscle pain after one week of simvastatin, at which time an MRS scan was repeated. MRS results identified several changes, most notably an increase in phosphocreatine recovery time (Figure 2) – indicative of impaired oxidative phosphorylation.

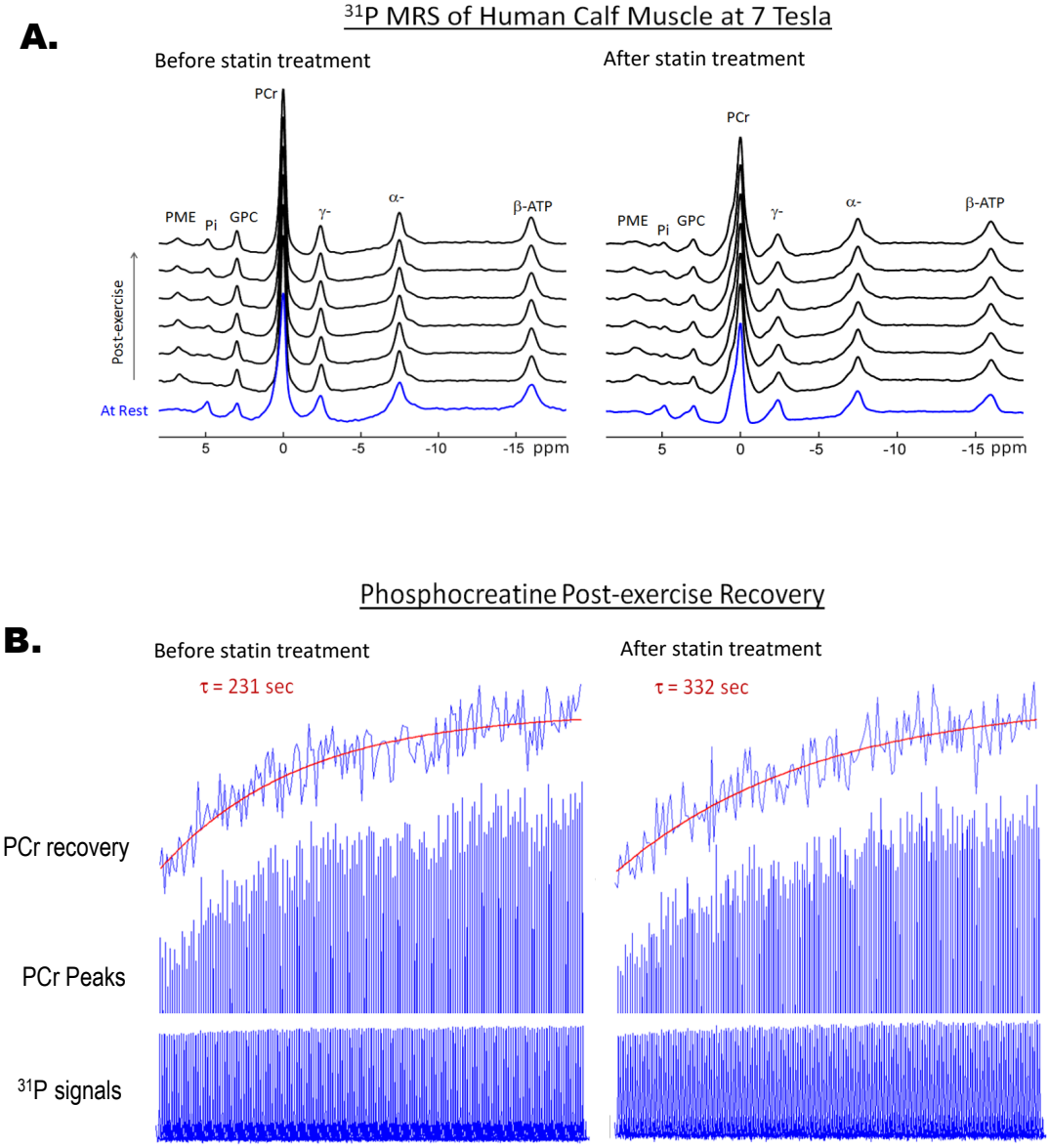
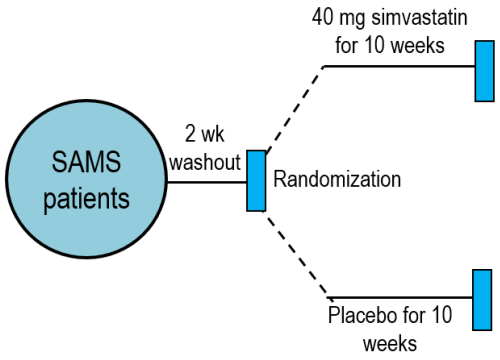


Figure 2: A. Representative series of ^{31}P signals acquired from calf muscle of a 65 yr old female SAMS patient at rest (blue traces) and after 2 min calf-raise exercise (black traces) prior to taking statin (left) and at one week of simvastatin 40 mg daily (right). Each ^{31}P spectrum represents a 2 min scan. Note that the magnitude of Pi signal was greatly reduced immediately after exercise and gradually recovered to its level at rest during post-exercise muscle relaxation. There is also a significant increase in PME signal after exercise, which is slowly attenuated with muscle relaxation. **B**. The full series of 180 phosphocreatine (PCr) ^{31}P signals acquired from the same patient prior to taking statin (left) and after one week of simvastatin 40 mg daily (right), showing the slowed oxidative phosphorylation due to simvastatin. The middle row represents zoomed PCr signals at the peak of ^{31}P signals. Top row represents the fitted PCr recoveries (red trace). Each PCr signal represents a single scan with sequence repetition time of 4 sec. Abbreviations: ATP, adenosine triphosphate; PCr phosphocreatine; GPC, glycerophosphocholine; Pi, inorganic phosphate; and PME, phosphomonoester including phosphocholine, phosphoethanolamine and sugar phosphates.



	Screening	Visit 1 (baseline)	Visit 2
Informed Consent	X		
Muscle/Pain Questionnaires	X	X	X
Blood draw	X	X	X
7T MRS		X	X
Muscle Biopsy			X

Figure 3: Study design

1.f M*ethodology, potential problems.*

Overall Design of the Study. This is a double-blind randomized, placebo-controlled study of simvastatin 40 mg daily vs placebo in SAMS patients. All enrolled subjects will be recruited from lipid specialty clinics and will undergo a washout of lipid lowering drugs followed by simvastatin 40 mg daily or placebo challenge for a maximum of 10 weeks (Figure 3). Visit 2 will occur either at 10 weeks after visit 1 or at the time muscle symptoms manifest, whichever occurs first.

This study design is based on the most robust clinical trial of SAMS patients (38). This study included a double-blind, placebo-controlled statin rechallenge in SAMS patients. Patients were challenged with either statin or placebo for 10 weeks in each arm of the study.

Choice of Study Drug. We chose simvastatin 40 mg daily since it is drug and dose most associated with SAMS (2). From each blood draw, we will measure serum levels of simvastatin (lactone and acid) as described previously (16).

Endpoints and Sample Size. The primary endpoint will be the differences in pre- and post-phosphocreatine relaxation time in SAMS patients on simvastatin compared to placebo. We chose 15 patients/arm since this sample size is within the range of prior pilot and feasibility studies (39).

Patient Recruitment. Table 1 outlines inclusion and exclusion criteria for SAMS patients. We will recruit from existing cohorts (40, 41), and we continue get new SAMS consults in our lipid specialty clinics. We expect to be able to recruit 1 SAMS patents and 1 control per month. Patients will get $100 per MRS study and $250 per muscle biopsy.

Table 1. Inclusion and exclusion criteria for SAMS patients

Inclusion Criteria	Exclusion Criteria
Adults, age > 18 ys or < 80 yrs	Patient who drink large quantities of grapefruit juice (> 1 quart daily).
Patients reporting complaints of statin-associated muscle symptoms, aches, weakness, cramps, or stiffness in the legs.	Patients on the following drugs for which the FDA has issued restrictions for using simvastatin 40 mg daily do to an increased risk of severe muscle injury such as itraconazole, posaconazole, ketoconazole, erythromycin, clarithromycin, telithromycin, HIV-1 protease inhibitors, nefazodone, gemfibrozil, cyclosporine, danazol, amiodarone, amlodipine, ranolazine, and verapamil.
	Patients with muscle-related pain that is not related to statin-use (e.g. muscle aches from strain or trauma) or remains unexplained.
	Any patients with underlying non-statin related muscle disorders.
	Presence of any clinically significant uncontrolled endocrine disease known to influence serum lipids or lipoproteins.
	Conditions of severe acute vascular stress (acute coronary syndrome, ischemic stroke, or major vascular surgery) within prior 3 months.
	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, transaminitis, or allergic reactions.
	History of fibromyalgia or rheumatologic disease with symptoms that may be confounded with statin-related muscle complaints.
	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.
	Pregnant or breast-feeding women. Statins are teratogenic, and the effects of high magnetic fields on a fetus are unknown.
	Women of reproductive age not on effective contraception. Adequate contraceptive measures include intrauterine device (IUD); bilateral tubal ligation; condom or diaphragm plus either contraceptive sponge, foam or jelly.
	Any person with implanted metal, because of MRS safety.
	Use of any active investigational drugs within 1 month or 5 half-lives, whichever is longer.
	History of antibodies to HMGCoA.

MRS Protocol. The MRS protocol has been previously described (35, 36). MR spectra will be measured in the 7T MRI scanner (Achieva, Philips Healthcare, Cleveland, OH).

For in-magnet exercise to elicit depletion of phosphocreatine (PCr) from calf muscle, the subject will be positioned supine and feet-first with the thickest part of the calf positioned in the center of the detection coil and secured by Velcro and a thick cushion pad. The ball of the exercising foot will be in natural contact to the MRI-compatible sand bags which will be fixed on a supporting board attached to MRI bed. The sand bags will provide a resistance to the force exerted by the exercising foot.

The subject will be asked to do exercise after a 2-min baseline 31P MRS scan (TR 4 sec), the exercise will be performed for 90 sec with plantar flexion at a frequency of 4 second by repetitively pushing on and releasing from the sand bags. The pushing duration will be 3 seconds and the releasing 1 second. The subject will be instructed to use maximal force when pushing. After the 90-sec exercise, the subject will be asked to relax but stay still while the scan continues. The post-exercise scan will last for 15 min.

The total scan session, including coil setup, subject preparation/positioning, 31P MRS data acquisition, and subject release from the scanner room, will be ~45 min. If a second bout of exercise is conducted, the scan session will be ~ 1 hr.

Muscle Biopsy Specimens. As described previously (42), muscle tissue will be obtained from the quadriceps muscles, specifically the *Vastus lateralis,* using the percutaneous needle biopsy technique. *The average yield should be 150 +/- 20 mg.* Specimens will be divided as follows: 50 mg will be required to isolate mitochondria and measure oxygen consumption rates (OCRs), 20 mg for electron microscopy studies, and 15 mg for RNAseq studies. The remainder of the sample will be flash frozen at -80°C for potential future studies.

We will isolate mitochondria from fresh muscle biopsy specimens as previously described (42). Muscle homogenates containing equivalent amounts of mitochondria will be distributed into Seahorse analyzer plates, and OCRs will be measured as described (43) using a Seahorse Xfe96 instrument (Seahorse Bioscience, Billerica, MA, USA). Transmission electron microscopy will be done by the UT Southwestern Electron Microscopy Core Facility as described previously (44). Gene expression studies will be done and analyzed by the UT Southwestern McDermott Center core facilities as previously described (45).

Challenges and Potential Problem Areas and Alternative Tactics.

Difficulty with recruiting. If this happens we would expand recruitment to other clinics at UT Southwestern – such as cardiology and primary care - and affiliated private practices.
Mitochondrial function not impaired. We will rely on gene expression data to identify pathways of interest.
P MRS data inconclusive. We would turn to other data that can be obtained from the studies: lactate dynamics and or intramyocellular lipids as measured by ^{1}H MRS signals.

C3. Timetable – Table 2

Table 2. TIMETABLE

AIMS/TASKS	Month 1-6	Month 6-12	Month 12-18	Month 18-24
Patient Recruitment	X	X	X	
MRS Studies	X	X	X	
OCRs	X	X	X	
Electron Microscopy	X	X	X	
Gene Expression				X
Data analysis				X
Manuscript Preparation				X

2. Significance of Problem to Cardiovascular or Stroke Research

2.a ***How will scientific knowledge or clinical practice be impacted if successful?***

Our contribution here is expected to be an **improved understanding** of what changes occur in muscle mitochondria after SAMS patients take statins. Improved mechanistic insight may lead to potential therapeutic interventions targeted at relieving muscle symptoms.

With respect to *outcomes*, we expect the MRS studies in this proposal will provide **preliminary data** to design a larger randomized, double-blind placebo controlled study of simvastatin in SAMS patients. Such a study will validate this technique as a diagnostic tool for SAMS.

2. b Will there be an impact on the methods/concepts/technologies?

We will improve future studies of SAMS patients. Several prior studies of SAMS patients involve statin rechallenges, but these studies have not improved understanding of SAMS (38, 46, 47). One major issue limits their interpretation: none included any objective measure of SAMS, relying instead on self-reported symptoms. The work proposed here will lead to improved clinical trial design. Specifically, this work will lead to objective markers (e.g. MRS signals) that can be used to select appropriate patients and follow their response to therapy.

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