Skeletal muscle mitochondrial capacity in patients with statin-associated muscle symptoms (SAMS)

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ABSTRACT

Objective The objective of this article is to evaluate near-infrared spectroscopy (NIRS), a non-invasive technique to assess tissue oxygenation and mitochondrial function, as a diagnostic tool for statin-associated muscle symptoms (SAMS).

Methods We verified SAMS in 39 statin-treated patients (23 women) using a double-blind, placebo-controlled, cross-over protocol. Subjects with suspected SAMS were randomised to simvastatin 20 mg/day or placebo for 8 weeks, followed by a 4-week no treatment period and then assigned to the alternative treatment, either simvastatin or placebo. Tissue oxygenation was measured before and after each statin or placebo treatment using NIRS during handgrip exercise at increasing intensities of maximal voluntary contraction (MVC).

Results 44% (n=17) of patients were confirmed as having SAMS (11 women) because they reported discomfort only during simvastatin treatment. There were no significant differences in percent change in tissue oxygenation in placebo versus statin at all % MVCs in all subjects. The percent change in tissue oxygenation also did not differ significantly between confirmed and unconfirmed SAMS subjects on statin (−2.4% vs −2.4%, respectively) or placebo treatment (−1.1% vs −9%, respectively). The percent change in tissue oxygenation was reduced after placebo therapy in unconfirmed SAMS subjects (−10.2%) (p≤0.01) suggesting potential measurement variability.

Conclusions NIRS in the forearm cannot differentiate between confirmed and unconfirmed SAMS, but further research is needed to assess the usability of NIRS as a diagnostic tool for SAMS.

Trial registration number NCT03653663.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Near-infrared spectroscopy (NIRS) is a non-invasive technique of assessing tissue oxygenation and mitochondrial function. Several mechanisms may contribute to statin-associated muscle symptoms (SAMS) including mitochondrial dysfunction.

WHAT THIS STUDY ADDS

⇒ This is the first randomised controlled cross-over trial to evaluate NIRS as a diagnostic tool for SAMS.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ NIRS in the forearm cannot differentiate between confirmed and unconfirmed SAMS, but further research is needed to assess the usability of NIRS as a diagnostic tool for SAMS. Further, the possibility that statins reduce mitochondrial function and tissue oxygenation should continue to be examined in larger patient populations.

INTRODUCTION

Statins are the most frequently prescribed drugs in the USA and world.1 They have few side effects but can produce statin-associated muscle symptoms (SAMS) consisting of myalgia or pain, cramps and weakness. SAMS reduce medication compliance and quality of life.2 SAMS are difficult to diagnose since there are no clinical predictors or validated biomarkers, and 30%–50% of patients on statins with self-reported SAMS have non-reproducible muscle complaints probably unrelated to statins.3 In an internet survey, approximately 60% of patients who discontinue statins report SAMS as the primary reason for discontinuation.4 The absence of predictors and diagnostic markers for SAMS makes its diagnosis and management difficult, forcing clinicians to rely on clinical complaints and drug cessation to make the diagnosis. Moreover, the absence of validated methods to diagnose and confirm SAMS has public health implications because patients stopping statins have an increased risk of cardiovascular events5 and higher healthcare costs.6

Several mechanisms may contribute to SAMS including mitochondrial dysfunction. Statins inhibit the mevalonate pathway thereby reducing cholesterol production but also reducing compounds important for normal mitochondrial function.7 Statins may also interfere with mitochondrial calcium metabolism,8 reduce mitochondrial respiratory chain components including coenzyme
Q10 and induce mitochondrial apoptosis. Thus, assessment of mitochondrial dysfunction may be useful in diagnosing SAMS.

Near-infrared spectroscopy (NIRS) is a non-invasive technique of assessing tissue oxygenation and mitochondrial function. NIRS during incremental handgrip exercise has a sensitivity and specificity of 91% for diagnosing metabolic myopathy in patients with mitochondrial disease. There are no randomised controlled cross-over studies to evaluate NIRS after incremental handgrip exercise as a diagnostic tool for SAMS. Thus, the present study sought to evaluate NIRS in patients with and without SAMS to determine its utility to diagnose SAMS.

METHODS
Study overview
We conducted a randomised, double-blind, placebo-controlled, cross-over trial to determine if NIRS could diagnose SAMS. Patients with muscle complaints during statin therapy were recruited from the Cholesterol Management Center at Hartford Hospital, a referral centre for patients with possible SAMS. Participants stopped their statins for at least 4 weeks before undergoing baseline testing. Baseline tissue oxygenation was measured using NIRS during a short handgrip exercise protocol. Subjects were then randomly assigned in a double-blind fashion to treatment with simvastatin 20 mg daily or matching placebo for 2 months (figure 1). The protocol allowed patients to end participation early if they developed intolerable muscle pain, yet none did so. Participants were called weekly to assess symptoms of muscle pain and to complete the Brief Pain Inventory (BPI). NIRS was repeated at the end of the first treatment period. Subjects then entered a 4-week washout, had repeat NIRS and received the alternate treatment: subjects first assigned to statin first crossed over to placebo and vice versa. NIRS was again repeated at the end of the second treatment period. Simvastatin and placebo were placed into identical capsules by a specialty pharmacy. Simvastatin tablets were obtained from a single supplier, fit into opaque capsules and covered with lactose. Placebo tablets were filled with lactose alone.

Study participants
We evaluated 129 possible participants. Participants were recruited if they were ≥40 years of age with a history of muscle complaints consistent with SAMS. Recruitment began in April 2018 and data collection terminated in December 2018 after predetermined sample size was obtained. We did not exclude individuals with a history of coronary artery disease, peripheral vascular disease, or an elevated creatine kinase (CK) <10 upper normal limit on statin treatment. Participants maintained a lipid-lowering diet during the study. Patients were not involved in the design and conduct of this research.
Sample size calculation
Our sample size was determined based on previous data regarding a NIRS technique\(^\text{12}\) that indicated that at low-intensity exercise, healthy controls had a 20±10% increase in tissue oxygenation versus 10±10% observed in patients with metabolic myopathies. We anticipated the expected difference in patients with and without SAMS on statin therapy, relative to placebo, to be 10% (SD of 10%), requiring 17 subjects in each group to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The type I error probability associated with this test of this null hypothesis is 0.05. Accordingly, we planned to enrol 40 subjects to facilitate data collection in approximately 20 in each group, accounting for potential subject dropouts.

Randomisation
A study randomisation code was obtained from Randomization.com (a Tufts Medical Center program) and participants were randomised to simvastatin and placebo in alternating, balanced order. The study physician generated a study log-in sheet where recruited subjects were enrolled, logged in and their study number assigned. After each vial was dispensed to the patient, the date of drug release was recorded. The entire study was unblinded once the last subject completed the protocol.

Muscle pain
The BPI (Short Form) was used to measure the location(s) and intensity of participants’ muscle pain, as well as the extent to which the muscle pain interfered with daily functioning, during the simvastatin and placebo treatments.\(^\text{13}\) A pain severity score was calculated by averaging scores on four pain-intensity items, and a pain interference score was calculated by averaging scores on seven pain-interference items. Participants who reported new or increased myalgia, cramps or muscle aching that persisted for 1 week (or was intolerable) were considered to have muscle symptoms during that study phase. Only participants who reported muscle pain on statin only were considered to have confirmed SAMS.

Serologic markers
Creatinine and thyroid stimulating hormone levels were measured at baseline. Serum lipids, CK, vitamin D and alanine aminotransferase levels were measured at each testing point.

Medication compliance assessment
Participants were instructed to take one study pill every day and to take the pill the next day before noon if they missed a day. Participants were queried about missed pills and problems taking medications during weekly phone calls. Participants returned unused pills after each portion of the study.

Muscle oxygenation to handgrip exercise
Skeletal muscle tissue oxygenation was measured with NIRS (Hamamatsu NIRO-200NX, Hamamatsu Photonics, Japan). The probe was positioned longitudinally on the forearm. Measurements were made of probe placement relative to the humerus, elbow, radius and ulna and recorded to ensure uniform probe placement in each subject throughout the study.

Maximal voluntary contraction (MVC) force in Newtons (N) was determined in the dominant arm using a digital handgrip dynamometer (Zonaphus, Boise, Idaho, USA) preceding each NIRS test. A pneumatic cuff (Hokanson Medical Electronics; Bellevue, Washington, USA) was placed around the ipsilateral upper arm. Subjects performed the exercise protocol seated. The protocol began by occluding the forearm arterial supply by inflating the cuff to 260 mm Hg until a steady state in deoxy[Hb+Mb] was reached. After the occlusion, there was a minimum 5-min rest period before baseline conditions were reached again and the incremental handgrip task was initiated. This task consisted of 2-min periods during which incremental cyclic forearm muscle contractions were performed at the rate of 1/2 Hz (1 s contraction, 1 s relaxation). The contractions were performed at 20%–60% of MVC. The contraction periods were separated by a 60 s of rest. The work intensity was initiated at 20% MVC and increased by 10% MVC at each step. This protocol continued until exhaustion and the subjects were unable to produce the required force. A smoothing procedure was performed in which the 10 s mean values were determined by means of a moving average. The amplitude of the deoxy[Hb+Mb] response during arterial occlusion was used as an index for each patient’s maximal \(\text{O}_2\) extraction and was set at 100%. The changes in deoxy[Hb+Mb] during each work rate were then expressed relative to this amplitude.\(^\text{12}\) We did not measure subcutaneous adipose tissue thickness at the site of the NIRS measurement. This is a possible study limitation because adipose tissue can affect the NIRS measurement.\(^\text{14}\)

Statistical methods
The primary analysis examined the % increase in deoxy[Hb+Mb] during the handgrip protocol between patients who did or did not have confirmed SAMS. Continuous baseline variables were summarised by the median and IQR, and comparisons between groups were performed using a one-way analysis of variance (ANOVA). Univariate comparisons of categorical data were performed using Fisher’s exact test. A repeated measures ANOVA with an autoregressive variance–covariance structure (to account for decreased correlation between lowest intensity and highest intensity measurements) was used to compare the % increase in deoxy[Hb+Mb] during the handgrip, modelled as a change score between statin and placebo conditions, in patients with and without confirmed SAMS. When a significant main effect was found, post hoc analysis was performed by means of an independent samples
t-test with Bonferroni correction for multiple comparisons to maintain the family-wise error rate. Relevant covariates were examined as either between-participant factors with interaction terms in the ANOVA model or as continuous predictors in an analysis of covariance model. Data are presented as mean±SD, 95% CI.

RESULTS
Baseline characteristics and SAMS outcomes
A total of 45 participants were randomised to simvas-tatin or placebo, of whom 39 provided complete data for analysis. No participants stopped their participation in the study prematurely due to intolerable muscle pain. Six participants did not provide sufficient NIRS data to be included in the final analysis. The participants had a mean age of 65±6 (mean±SD). Among the 39 patients, 17 (44%) patients were confirmed as having SAMS, whereas 22 (56%) had unconfirmed SAMS; 1 patient (3%) had pain both on placebo and statin, 7 patients (18%) experienced pain only on placebo and 14 patients (36%) had no symptoms on placebo and statin (table 1). Baseline characteristics did not differ between confirmed and unconfirmed SAMS subjects (table 2). Pills were counted at each visit to assess medication compliance. Patients were 100% compliant. Notably, patients treated with simvastatin showed the expected reductions (−33%) in LDL-C levels (online supplemental table 1).

Oxygen extraction
The percent change in tissue oxygenation did not differ between the placebo and statin treatments at all % MVCs in the total sample (figure 2). Tissue oxygenation decreased after both placebo (−6.24±33.65, 95% CI −11.1 to −1.5) and statin treatment (−2.4±26.7, 95% CI −6.1 to 1.4), but these decreases were not statistically significant (figure 3A). Moreover, the percent change in tissue oxygenation did not differ between subjects with confirmed and unconfirmed SAMS during statin treatment (−2.4% vs −2.4%, respectively) or placebo treatment (−1.1% vs −9%, respectively) (figure 3B). However, tissue oxygenation was significantly reduced after placebo therapy in unconfirmed SAMS subjects (−10.2±39, 95% CI [−17.6 to −2.85], p=0.008, R²=0.03), suggesting that these changes could be attributed to measurement variability.

Creatine kinase
The change in CK levels from pretreatment to post treatment did not show significant differences between the

placebo (−9.5±33.91, 95% CI −21.6 to 2.1) and statin (−16.91±57.66, 95% CI −36.7 to 2.9) treatments in the total sample (online supplemental figure 1A). Similarly, the prechange to post change in CK also did not significantly differ between subjects with confirmed (−33.2 to −3.1) and unconfirmed (−37.2 to 8.6) on statin treatment or subjects with confirmed (−21.6 to 2.1) and unconfirmed SAMS (−18±33.1, 95% CI −33.2 to −3.1) on placebo treatment (online supplemental figure 1B).

Brief Pain Inventory
The average pain severity experienced during daily life was significantly higher with statin therapy compared with placebo in the total sample (1.15±1.76 vs 0.98±1.46) (p=0.04). Moreover, BPI was higher during statin therapy in subjects with confirmed SAMS (1.75±0.6) than in subjects with unconfirmed SAMS (0.66±0.21) (p≤0.01).

DISCUSSION
We sought to determine the utility of skeletal muscle NIRS to diagnose SAMS. This is the first study to our knowledge to investigate changes in tissue oxygenation during handgrip exercise in patients who reported statin intolerance due to muscle pain. Among the 39 possible

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<thead>
<tr>
<th>Table 1</th>
<th>Statin-associated muscle symptoms (SAMS) outcomes</th>
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<tr>
<td>SAMS outcomes</td>
<td>Participants (%)</td>
</tr>
<tr>
<td>Confirmed SAMS (pain on statin only)</td>
<td>17/39 (44)</td>
</tr>
<tr>
<td>Unconfirmed SAMS (pain on placebo only)</td>
<td>7/39 (18)</td>
</tr>
<tr>
<td>Unconfirmed SAMS (pain on placebo and statin)</td>
<td>1/39 (3)</td>
</tr>
<tr>
<td>Unconfirmed SAMS (no symptoms)</td>
<td>14/39 (36)</td>
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<tr>
<th>Table 2</th>
<th>Participant baseline characteristics in confirmed versus unconfirmed SAMS subjects</th>
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<tbody>
<tr>
<td>Baseline participant characteristics (n=39) (median (IQR))</td>
<td>Confirmed SAMS (n=17)</td>
</tr>
<tr>
<td>Age</td>
<td>70 (15)</td>
</tr>
<tr>
<td>% Female</td>
<td>65</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.4 (8.4)</td>
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<tr>
<td>Weight (kg)</td>
<td>77.2 (10)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>28.1 (5.5)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>98 (10)</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>134 (16)</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>68 (8)</td>
</tr>
<tr>
<td>Pulse rate</td>
<td>65 (21)</td>
</tr>
<tr>
<td>Average handgrip (kg)</td>
<td>33 (12)</td>
</tr>
<tr>
<td>Max handgrip (kg)</td>
<td>36 (15)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>229 (52)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>54 (28)</td>
</tr>
<tr>
<td>LDL-C</td>
<td>147 (46)</td>
</tr>
<tr>
<td>CK (mg/dL)</td>
<td>81 (61)</td>
</tr>
<tr>
<td>Occlusion time (min:s)</td>
<td>0.35 (0.10)</td>
</tr>
</tbody>
</table>

CK, creatine kinase; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SAMS, statin-associated muscle symptoms; SBP, systolic blood pressure.
Aortic and vascular disease

SAMS patients, we confirmed SAMS in 17 patients (44%). However, there was no difference in tissue oxygenation between the statin and placebo treatments in the entire cohort (n=39) of possible SAMS patients. Further, there were no differences in tissue oxygenation between the confirmed and unconfirmed SAMS subjects, indicating that NIRS could not distinguish patients with confirmed SAMS from patients with non-specific muscle complaints. However, the significant reductions observed in tissue oxygenation after placebo treatment in patients with non-specific muscle complaints underscore the importance of considering measurement variability in interpreting these results. Further investigations may help elucidate the factors contributing to these fluctuations in tissue oxygenation and their potential implications for SAMS diagnosis and management.

The mechanisms mediating statin myopathy are unclear, but interest has focused on mitochondrial dysfunction. Patients with mitochondrial myopathies exhibit lower venous $O_2$ desaturation during exercise, as observed by blunted increases in deoxy[Hb+Mb]. Reduced $O_2$ desaturation reflects limited capacity to increase microvascular $O_2$ extraction and thus an altered muscle blood supply to oxygen demand relationship. To observe these changes, we used a simple and safe handgrip exercise protocol that requires forearm arterial occlusion. We used muscle in the forearm because occlusive cuffs can be easily applied above the elbow. The hyperaemic response following cuff release provides a maximal, relative deoxygenation value which limits the potential of confounders (eg, adipose tissue) derived from absolute values. Our subjects, all of whom had muscle complaints associated with statins, demonstrated non-significantly lower increases in deoxy[Hb+Mb] regardless of treatment with statin or placebo. These small reductions in deoxy[Hb+Mb] are not comparable to patients with mitochondrial myopathies.

Furthermore, we determined whether NIRS could differentiate between patients with confirmed SAMS and those with unconfirmed SAMS (ie, non-specific muscle complaints). Only patients with unconfirmed SAMS exhibited significant reductions in tissue oxygenation during placebo; these differences are likely due to methodological variation. These findings suggest that NIRS cannot differentiate confirmed from unconfirmed SAMS and will not be useful in diagnosing SAMS in symptomatic subjects. Our findings agree with recent research that investigated NIRS in symptomatic and asymptomatic patients.

Figure 2  The percent increase in tissue oxygenation during handgrip exercise. The group means±SD of percent increase in tissue oxygenation (near-infrared spectroscopy) deoxy [Hb+Mb] (%) for the different maximal voluntary contraction (MVC) (20%–60%) during handgrip exercise in all patients.

Figure 3  The percent increase in tissue oxygenation during handgrip exercise. The group means±SD of percent increase in tissue oxygenation (near-infrared spectroscopy) deoxy [Hb+Mb] (%) for average 20%–60% maximal voluntary contraction (MVC) during handgrip exercise. (A) In all patients for preplacebo and statin versus postplacebo and statin. (B) In confirmed versus unconfirmed patients on statin (CONFIRMED-STA, UNCONFIRMED-STA) versus placebo (CONFIRMED-PLA, UNCONFIRMED-PLA) therapy. *P=0.04. CONFIRMED-PLA, symptoms present on placebo; CONFIRMED-STA, symptoms on statin; UNCONFIRMED-PLA, no symptoms present on placebo; UNCONFIRMED-STA, no symptoms on a statin.
statin users after exercise. That study measured NIRS using the gastrocnemius muscle before a moderate-intensity, 5-day walking intervention. The recovery of muscle oxygen consumption was similar in symptomatic statin users compared with control subjects after exercise. NIRS may not be sensitive enough to differentiate SAMS patients from patients with non-specific muscle complaints.

Study limitations

Our study has several important limitations. Our sample size was small and could have missed subtle changes in mitochondrial function. Tissue oxygenation was lower after statin therapy in confirmed SAMS subjects but was also reduced after placebo therapy in unconfirmed SAMS subjects suggesting that these changes were due to measurement variability. We did not measure subcutaneous adipose tissue thickness at the site of the NIRS measurement. This is a possible study limitation because adipose tissue can affect the NIRS measurement. Statin symptoms typically affect large proximal muscle groups. We measured the response of forearm muscles using because incremental handgrip exercise protocol is easier to perform in patient population; we could have missed statin-induced changes if they occurred in large muscle groups. We only examined the effect of simvastatin on muscle complaints and tissue oxygenation. We selected a low dose because most patients with SAMS are unwilling to participate if we use high-dose statin treatment. Simvastatin at a higher dose or other statins at various doses might produce different results. Patients had stopped their statin regime at least 4 weeks before baseline measurements. This period may be too short to totally eliminate the effects of statins on mitochondrial function and therefore may have masked the effect of statins on mitochondria measured with NIRS. Our study lasted only 8 weeks, which may have been insufficient to alter mitochondrial metabolism, but most studies suggest that SAMS occur with several weeks of initiating statin treatment. All of our patients were Caucasian and non-Hispanic limiting the generalisability of our results.

CONCLUSIONS

This study, to our knowledge, is the only randomised, double-blind, placebo-controlled, cross-over trial conducted to determine the utility of NIRS after exercise to diagnose SAMS. NIRS did not distinguish patients with confirmed SAMS from patients with non-specific muscle complaints. However, tissue oxygenation was significantly reduced after placebo therapy in unconfirmed SAMS subjects suggesting that these changes could be attributed to measurement variability. NIRS in the forearm cannot differentiate between confirmed and unconfirmed SAMS, but further research is needed to assess the usability of NIRS as a diagnostic tool for SAMS. Continued efforts should be made to find more sensitive clinical predictors or validated biomarkers for identifying SAMS in clinical practice. Further, the possibility that statins reduce mitochondrial function and tissue oxygenation should be examined in larger patient populations.

Acknowledgements We thank Gregory Panza, Amanda Zaleski and Adam Lepley for their assistance with data acquisition.

Contributors BT, PT, CMW and OSK conceived and designed the study. LM drafted the manuscript. PT and OSK undertook critical review. BT and RS acquired and organised the data. LM, OSK and SGN carried out data analysis. BT, RS, CMW and PT provided administrative, technical or material support. BT obtained funding. All authors have read, reviewed and approved this manuscript for submission. LM is the guarantor of the overall content of this submission.

Funding CONS01037-Hatch Capacity Grant, 17GNT 33661247, Grant-In-Aid, National Institute of Food and Agriculture (NIFA) 10/012020-09/30/2023.

Competing interests Hartford Hospital through Dr. Thompson has received research funds from Norvalis and Esperion for studies of lipid metabolism.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval This study involves human participants and was approved by Hartford Healthcare Institutional Review Board (E-HHC-2017-0134). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The data underlying this article will be shared on reasonable request to the corresponding author.

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