University of Kansas Medical Center
RESEARCH PROTOCOL INVOLVING HUMAN SUBJECTS

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Principal Investigator: John Thyfault

Study Title: Impact of statin therapy on muscle mitochondrial function and aerobic capacity

Co- Investigator(s): Sandra Billinger, Jianghua He, Daniel Parente

I. Purpose, Background and Rationale

A. Abstract

More than 40 million Americans are currently take statins for the treatment or prevention of hyperlipidemia and cardiovascular disease (CVD). Based on the new usage guidelines from the American College of Cardiology and the American Heart Association (ACC/AHA) (2), that number is expected to increase to over 60 million (3), reflecting the growing sentiment for more wide-spread use of statins, even in otherwise asymptomatic patients. Although generally well-tolerated, statin therapy is not without risks. The most common reported side effects include mild to moderate muscle weakness, fatigue and/or pain, the incidence of which increase as a function of both dose and duration of statin use (4, 5). Statins also increase the risk of developing more serious metabolic conditions, including insulin resistance and type 2 diabetes (6-9). The underlying mechanism(s) for these complications is unknown. In recent years however, evidence has been mounting from both cell culture and animal models that statins interfere with mitochondrial function in muscle (10-12). In the present application, we provide data from both ex vivo and in vivo preliminary studies in humans suggesting that statins induce progressive and quite striking reductions in skeletal muscle mitochondrial respiratory function. The overall aim of the study is to determine the impact of low and high dose statin therapy on skeletal muscle mitochondrial function, insulin sensitivity, and cardiorespiratory fitness. Our hypothesis is that statin therapy produces a decline in mitochondrial respiratory function in skeletal muscle that is both dose and duration dependent, compromising metabolic and cardiorespiratory function.

B. Background and Significance

According to the Centers for Disease Control and Prevention, nearly one of every four adults over 40 years of age is taking a statin – which equates to more than 40 million Americans. This number is predicted to increase to over 60 million Americans (3) under the new guidelines released in 2013 by the American College of Cardiology and the American Heart Association (ACC/AHA). The new guidelines which emphasize prevention of stroke as well as cardiovascular disease (CVD), simplify evaluation by dividing patients into two broad risk categories, and feature a risk prediction algorithm (14). The new guidelines represent a departure from the 2002 National Cholesterol Education Program Adult Treatment Panel III statin therapy recommendations based on LDL-C treatment target levels of <70-100 mg/dl, depending on risk (15). Although not without controversy (16), the new algorithm recommends initiation of statin therapy for primary prevention in patients with a predicted 10-year risk of CVD of ≥7.5% and consideration of statin therapy for patients with 10-year risks of between 5-7.5%. Backed by a recent study spanning six continents showing a small but significant decrease in CVD events (17) the new guidelines are expected to lead to a dramatic increase in the use of statins

world-wide for primary prevention in middle-aged individuals who do not have, but are at risk of developing CVD.

Without question, statin therapy is extremely effective at lowering LDL-C and risk for CVD in high risk populations, and, in general, statins are well-tolerated (18). Muscle fatigue, pain and weakness are idiopathic but well known potential side effects. Less appreciated is the fact that statins also increase the risk of developing insulin resistance/type 2 diabetes (6-9, 19-21) and attenuate the beneficial effects of exercise on cardiovascular adaptations and fitness (13). In fact, statins when combined with exercise increase the risk and severity of adverse muscle reactions (22-24). This interaction effect is of obvious concern given the importance of physical activity in the clinical treatment of patients with type 2 diabetes and/or CVD. Although the mechanism(s) underlying the side effects of statins is unknown, there is evidence that the impact of statins on skeletal muscle may be progressive. In the only direct comparative cohort study between patients on statins and matched controls, duration of statin therapy (< 10 vs. ≥ 10 months) was found to increase the risk of developing muscle-related side effects (5), which is consistent with clinical reports of some patients experiencing symptoms only after years of statin therapy (25). Perhaps most alarming, mounting evidence, including preliminary data in this application, indicates that statins directly compromise mitochondrial respiratory function, providing a potential unifying mechanism for the dose- and duration-dependent complications associated with statin use. Clinically, because the loss of aerobic capacity with age (Fig. 1)(1) is an independent risk factor for morbidity and mortality (26,27) a direct inhibition of respiratory function induced by statin therapy would be expected to shift this relationship (see redline) and hasten the decline in aerobic capacity needed to support daily living (Fig. 1). This is especially alarming given that the greatest increase in statin prescription is predicted to occur in adults >60 years of age who already have low aerobic capacity(3) and that statin therapy is being widely advocated for younger individuals, meaning that lifetime statin use may potentiate aging induced loss of aerobic capacity (28). Thus, while the risk for CVD undoubtedly favors statin therapy for many patients, it is imperative to develop a better understanding of the mechanism(s) underlying the effect of statins on muscle biology to better define the risk – benefit ratio for all patients.

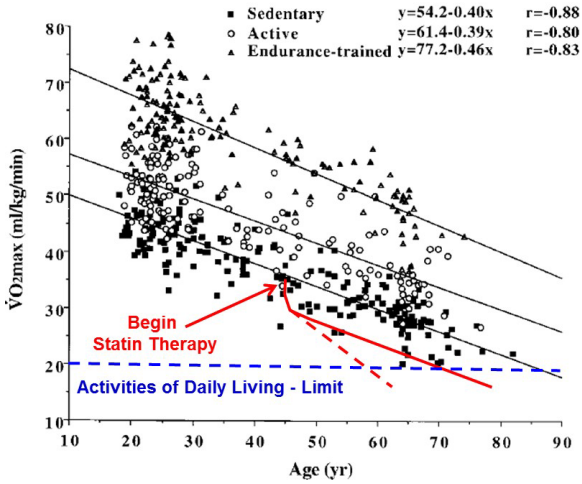


Fig 1. Relationship between maximal oxygen uptake and age in sedentary, active and endurance trained subjects. The figure is adapted from Wilson and Tanaka (1) and depicts the potential impact of statin therapy to shift (solid red line) or accelerate (dotted red line) the loss of aerobic capacity relative to age and therefore morbidity and mortality.

The widespread use of statins combined with the potential for more aggressive use of high intensity statin therapy emphasizes the urgency and importance of this research topic to the medical community. Importantly, we are studying one of the most widely prescribed and utilized statins, atorvastatin (trade name "Lipitor") (29) making the outcomes applicable to a large number of statin users. In addition, high dose (80 mg/day) atorvastatin is widely employed as the "go to" dose based on data from three clinical trials showing the largest effect in terms of lowering of CVD risk (30-32). This will immediately help physicians and patients make better-informed decisions, thereby improving clinical practice in the treatment of CVD and other disorders of metabolism.

C. Rationale and Preliminary Data

Statins block 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in de novo cholesterol biosynthesis. Several mechanisms have been proposed to account for the statin-induced changes in muscle function, including altered membrane properties due to modifications in the cholesterol/ phospholipid ratio (33), disrupted calcium cycling (34), endoplasmic reticulum stress-induced inflammatory response (35), decreased post-translational isoprenylation of small GTPase proteins such as Ras, Rho, and Rab (36, 37), and loss of ubiquinone, an essential component of the mitochondrial electron transport system (38). However, thus far evidence supporting each of these potential mechanisms has been circumstantial and/or inconclusive, and thus failed to account for statin-induced muscle dysfunction (36-40).

There is increasing evidence from both in vitro and in vivo studies that statins may interfere with mitochondrial function in muscle, although the mechanism(s) is ill defined, particularly in humans. Mitochondria generate and maintain the energy and redox charges that give life to cells – the vital forces that animate anatomy if you will. The overriding hypothesis of this project is that statin therapy induces a dose- and duration-dependent deterioration in skeletal muscle mitochondrial function, which, if substantiated, could offer a potential unifying explanation for the complications reported with statin therapy. Quite striking reductions in maximal ADP-stimulated respiration were first reported in permeabilized human skeletal muscle fiber bundles acutely exposed to simvastatin (11, 41); however, these experiments were conducted using statin concentrations >50 μM, which far exceeds the intramuscular concentration (~<2-5 μM) resulting from typical oral doses (20-80 mg) (42, 43). Using more physiologically relevant concentrations (1-5 μM), we recently reported that ADP- stimulated state 3 respiration is decreased by >30% in primary human skeletal myotubes within 48h of statin exposure (Fig. 2). The reduction in mitochondrial respiratory capacity occurred in the absence of any change in mitochondrial content or integrity (i.e., no change in citrate synthase activity or response to cytochrome c addition, respectively). Simvastatin exposure also induced myotube atrophy and apoptosis, suggesting altered mitochondrial function may be involved in the etiology of statin induced myopathic symptoms (44). These data are supported by previous clinical findings that simvastatin treatment in human patients lowered skeletal muscle mitochondrial DNA (mtDNA) a marker of mitochondrial content, by 50% in 8 weeks (45).

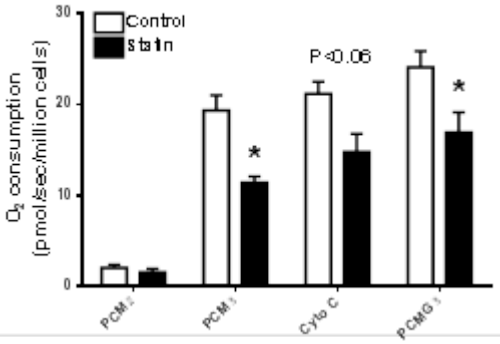


Fig 2. O_2 consumption rate in permeabilized primary human skeletal myotubes after 48h treatment with vehicle (DMSO) or statin (5 μM simvastatin). Measurements made during respiration supported by palmitoylcarnitine + malate under state 2 (-ADP, PCM_2), and state 3 (+ADP, PCM_3) conditions, and following addition of cytochrome c and glutamate ($PCMG_3$, complex I substrate). Data shows statin exposure depresses maximal ADP-stimulated O_2 consumption during respiration supported by multiple substrates. Lack of increase in O_2 consumption upon addition of cytochrome c is a quality control measure, demonstrating electron transport chain is intact. Data are mean ±SE. * P<0.05 vs. control. (42)

To examine the potential acute impact of statins on mitochondrial function in vitro, we obtained skeletal muscle biopsies from sedentary healthy humans (N=7, 25-50 years of age, 12h fasted, no history of elevated lipoproteins or statin use, normal liver function), and exposed permeabilized fiber bundles to vehicle (DMSO), simvastatin (2.5 μM) or atorvastatin (10 μM) for 10 min and throughout subsequent substrate additions (37°C). Maximal ADP-stimulated O_2 consumption rate (JO_2) during respiration supported by glutamate/malate (complex I substrate; Fig. 3A) or succinate (complex II substrate; Fig. 3B) plus rotenone (to block reverse electron flow) was ~30- 50% lower after acute exposure to either

statin compared with vehicle alone. These findings suggest that statins may directly interfere with mitochondrial respiratory function independent of their effect on mevalonate synthesis.

To examine the potential impact of statin therapy on skeletal muscle mitochondrial function in vivo, a preliminary repeated measures study was conducted on six sedentary participants (36.3 ±3.4 y, 31.9 ±0.7 BMI, VO_2peak=24.7 ±1.9 ml/kg/min) with no history of elevated lipoproteins or statin use.

After baseline testing (Day 0), participants began statin therapy (80 mg/kg/day atorvastatin) and were retested after 14, 28 and 56 days. Total cholesterol (mean ±SE) decreased from 182 ±27 to 123 ±16 mg/dl and LDL decreased from 120 ±26 to 64 ±15 mg/dl by day 56. Plasma creatine kinase, ALT and AST levels did not change indicating no skeletal muscle or liver damage. At the whole muscle level however, non-invasive determination of O_2 recovery kinetics in vivo by near infrared spectroscopy (NIRS) revealed a ~20% statin-induced decrease in maximal mitochondrial function (Fig. 4A). Specifically, note the decrease in the mVO_2 rate constant (Fig. 4A inset), which is directly related to mitochondrial respiratory capacity (46). At the level of the mitochondria in muscle, maximal ADP-stimulated respiration progressively decreased over the duration of statin therapy to ~50% of pretreatment values (Fig. 4B). This striking decrease was also evident in three other titration protocols using different substrates (data not shown). Importantly, addition of cytochrome c at the conclusion of each maximal respiration protocol failed to increase respiration, indicating that the mitochondria/ETS were intact and not otherwise damaged by the statin therapy. The capacity for mitochondrial calcium retention, an index of mitochondrial fragility, also decreased following statin therapy (Fig. 4C). Given the sensitivity of the permeability transition pore to calcium, this provides a potential mechanism by which statins may lead to collapse of respiratory function and eventually trigger apoptosis as we have previously observed in cultured human skeletal myotubes exposed to statins (44). Importantly, mitochondrial content, assessed by western blotting and citrate synthase activity, was not altered (data not shown). Interestingly, mitochondrial H_2O_2 emitting potential under two separate protocols was not altered by statin therapy (not shown). Finally, insulin sensitivity index (ISI), determined by IVGTTs, decreased by

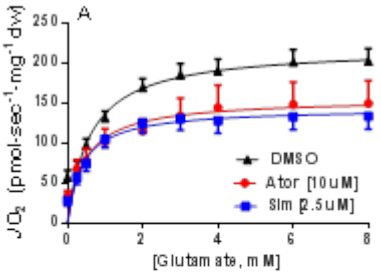


Fig 3. In vitro acute exposure to statins decreases mitochondrial respiratory capacity. See text for explanation.

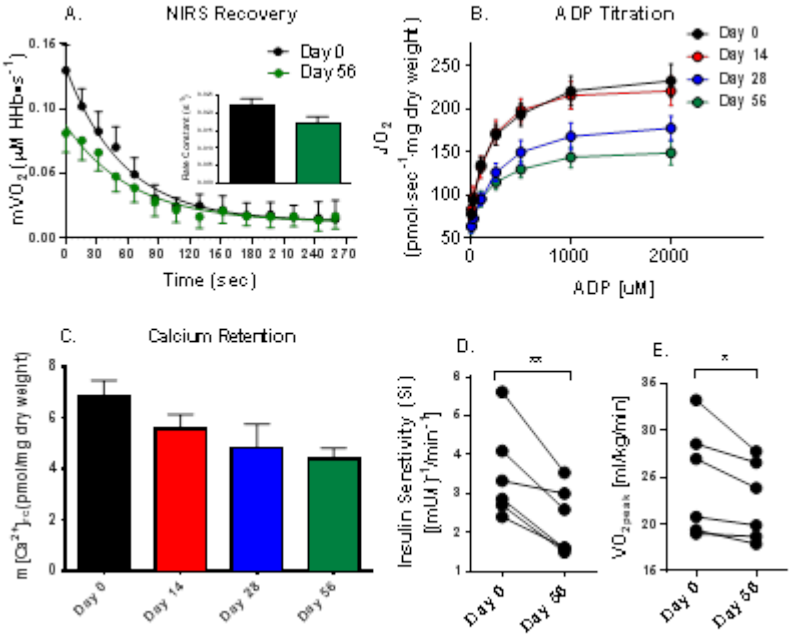


Fig 4. Statin therapy decreases skeletal muscle mitochondrial res
calcium retention and whole body insulin sensitivity. See text for

~34% (3.50 ±0.48 to 2.30 ±0.35 mU) and whole body VO_2 peak decreased by ~7% (24.68 ±2.38 to 22.47 ±1.72 ml/kg/min) as a result of the statin treatment (Fig. 4D).

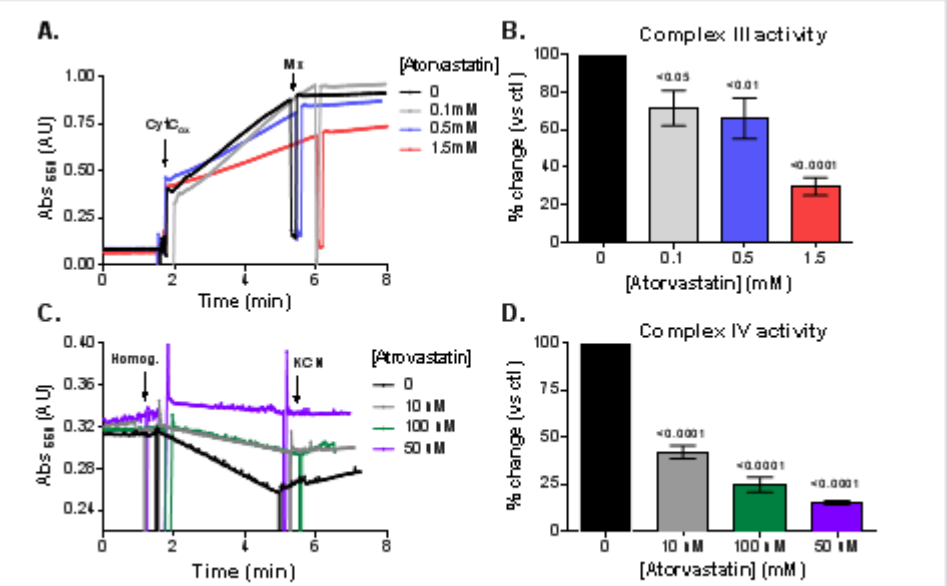


Fig 5. Atorvastatin dose response inhibition of mitochondrial complex III and complex IV performed on muscle homogenates from subjects prior to initiating statin therapy.

To further explore the potential mechanisms by which atorvastatin may be impairing mitochondrial function, the maximal enzymatic activity of each individual complex of the electron transport chain was determined in vitro using muscle homogenates from the same participants (prior to initiating statin treatment) in the presence of increasing concentrations of atorvastatin. Statin exposure did not affect complex I or II activity (not shown), but did inhibit complex III at mM concentrations (Fig. 5A & 5B), similar to a recent report (12). Strikingly, atorvastatin inhibited complex IV at concentrations as low as 10 nM (Fig. 5C & 5D), which equated to ~7.5 ng/g cell protein, similar to the concentration range (~5-18 ng/g protein) of atorvastatin detected by mass spec in muscle from participants undergoing the statin therapy. Sensitivity to statins has not previously been reported for complex IV, providing a potential mechanism for the compromised respiratory capacity observed in vivo (Fig. 4).

These findings are preliminary evidence that skeletal muscle mitochondrial respiratory capacity and function declines rapidly with high dose atorvastatin therapy. These findings are consistent with several factors that are likely to be at play simultaneously that affect the net effect of statins on mitochondrial and whole muscle function in vivo, emphasizing the need for a long-duration, dose-response study in humans. These factors include:

1. Intramyofiber statin concentration relative to the density of mitochondria in the myofiber over which the statin is distributed. Factors such as dosage, pharmacokinetics and half-life will determine the concentration and time course over which the statin is present in myofibers (43, 47). Factors determining mitochondrial density in skeletal muscle, which varies considerably among individuals, include genetics, percent fiber type distribution (i.e., type I, IIa and IIx/IIb), age, VO_2peak, and activity level (48). The acute impact of a given dose of statin on mitochondrial respiratory function therefore is most likely transient, driven by the intramuscular statin concentration relative to the mitochondrial density and the turnover rate of the statin.
2. Quality of the mitochondria at the time therapy is initiated. Patients with subclinical mitochondrial dysfunction may be predisposed to developing myopathic symptoms, or statin treatment may unmask or induce nascent mitochondrial dysfunction (49). Consistent with this notion, aging, diabetes, obesity, hypothyroidism, renal disease, and frailty are all associated with reduced mitochondrial function in muscle (50, 51), and increased risk of muscle-related adverse reactions to statins (22, 52-54).
3. Mitochondrial respiratory demand relative to intramyofiber statin concentration. Our preliminary data indicate that statin-induced inhibition of respiration increases as a function of ADP-

stimulated respiratory demand (Fig. 3 & 4). In other words, inhibition is negligible at low rates of respiration, but is clearly present as the demand for respiration increases. This could explain why exercise increases the risk of developing myopathic symptoms (23, 55) and why statins are not well tolerated by athletes (56).

4. Declining respiratory reserve capacity over time. Recent reports indicate patients may not present with myopathic symptoms until several years after initiating statin treatment (25). A slowly progressing decline in mitochondrial content as a result of aging and/or inactivity will decrease the density of mitochondria over which the statin is distributed (#1) and increase the relative respiratory demand on the remaining mitochondria (#3). This reduces the available reserve capacity, eventually reaching the point (i.e., threshold) where the decline in function imposed by the statin creates added demand to existing mitochondria that exceeds the reserve capacity and is sufficient to generate myopathic symptoms.

In summary, the goal is to determine if atorvastatin therapy induces a progressive decline in skeletal muscle mitochondrial respiratory function, insulin sensitivity, and cardiorespiratory fitness that is dose and duration dependent.

II. Research Plan and Design

A. Study Objectives: The overall aim of the study is to determine the impact of low and high dose statin therapy on skeletal muscle mitochondrial function, insulin sensitivity, and cardiorespiratory fitness.

a. Primary Objective:

i. <u>In Situ Mitochondrial Function:</u> Multiple aspects of mitochondrial function (basal and ADP-stimulated respiratory kinetics under multiple substrate combinations, H_2O_2 production and emitting potential) will be assessed in duplicate on permeabilized fiber bundles from freshly obtained muscle biopsy samples as previously described (61-64). Fibers are then freeze dried for determination of citrate synthase activity (index of mitochondrial content). Frozen muscle tissue will also be analyzed for electron transport complex activity (Fig. 5), content (Western blot using complex I through V antibody cocktail) and cellular redox state (GSH/GSSG, thioredoxinred/thioredoxinox) as previously described (63).Of the remaining approximate ≥90 mg of muscle biopsy tissue, a small portion (<5 mg) will be prepped for electron microscopy for future analysis of morphology (mitochondrial size, content, ragged muscle fiber analysis) (66). Remaining muscle will be frozen for later analysis of intracellular atorvastatin concentration (both the acid and lactone forms) (67), ubiquinone (co-enzyme Q10) (68, 69), and potential future gene array, metabolomics, or proteomics analysis.

b. Secondary Objectives:

i. <u>In Vivo Mitochondrial Function (NIRS):</u> Muscle oxygen consumption (mVO_2) and the recovery kinetics will be determined during a series of repeated arterial occlusions following maximal knee extension. The mVO_2 data are fit to a mono-exponential function to calculate the rate constant, which is directly related to the mitochondrial respiratory capacity.

ii. <u>Cardiorespiratory Fitness, Physical Function and Body Composition:</u> Whole body steady state submaximal O_2 consumption will be determined to examine VO_2 cost of exercise. VO_2 peak, peak heart rate, post exercise oxygen consumption and

heart rate will be determined as previously described (13). Creatine kinase will be measure following maximal exercise. DEXA will measure bone mineral content (BMC), bone mineral density (BMD) and body composition (fat mass, fat-free mass, and percent body fat).

iii. Pain Assessments, and Physical Activity : Pressure thresholds will be determined using an algometer during rest and after exercise. Current and usual whole body muscle pain while restingusing 0-10 numeric scale. Steps, physical activity and sedentary time will be determined over a 5-day period using pedometers and accelerometers.

iv. Fasting Blood Draw: Glucose, insulin, total cholesterol, HDL-C, LDL-C, triglycerides, creatinine, CK, and ALT will be measured in fasting blood samples.

v. Insulin Sensitivity: Glucose and insulin, and insulin sensitivity will be calculated using a minimal model program following the IVGTT.

vi. Cardiovascular Function: Heart rate and stroke volume (and finometer) will be measured under resting conditions.

B. Study Type and Design: This study is a longitudinal, repeated measures, double-blinded design with participants randomly assigned to placebo, low (20 mg/d) or high (80 mg/d) atorvastatin therapy for a one year dose-response study. We expect to screen approximately of 500 participants to enroll (randomize) a maximum of 60 participants. The goal is to have 45 participants complete all study procedures (15 in each group). Repeated measures design allows each subject to serve as their own control. Placebo groups are included to account for potential changes in muscle mitochondrial function independent of statin therapy over the 1 year dose-response.

C. Sample size, statistical methods, and power calculation: The randomization for study drugs will be carried out by the biostatistician using a computer-based random number generator at a 1:1:1 allocation. Sample size for study is powered on the basis of detecting a difference in mitochondrial respiratory function, the primary outcome measure, between three groups (e.g., placebo, and 20 or 80 mg/d atorvastatin therapy) at 12 months post-treatment. Our pilot study of statin effect on maximal ADP-stimulated O_2 consumption rate (JO_2) after 56 days based on 8 participants showed a mean change of 88.5 (pmol/sec/mg) with standard deviation of 59.9 (pmol/sec/mg), which can be standardized into a very large pre-post effect size of -1.48 (Cohen's d). All of these 8 participants experienced a decrease of 11.13% to 66.94% in 56 days, suggesting the decreasing trends are very consistent among participants. The overall decreasing trend of maximal ADP-stimulated O_2 consumption rate across all time points also suggests that the decreasing trend will continue beyond 56 days. For the other outcomes variables, insulin-sensitivity and in-vivo O_2 recovery kinetics (NIRS), only measurements at baseline and day 56 are available and the observed changes are -0.65 (1.20) $(mU/L)^{-1}$ min^{-1} and 19.07 (21.80) mVO_2, which can be standardized into effect sizes of -0.54, and 0.87, respectively. In a much longer study period of 12 month, the within group changes for these variables are expected to be greater. Expecting no change or a small change in the placebo as well as the low-dose group, our primary analyses are two subgroup comparisons: high-dose vs. low-dose and high-dose vs. placebo and the two expected between-group effect sizes (high-dose vs. low-dose and high-dose vs. placebo) are large (close to the within-group effect sizes in the pilot data). With 27 participants in each group, a two-sided two sample t-test has 80% power of detecting an effect size of 0.87 (Cohen's d) at 0.025 level to maintain the overall type I error rate at 0.05 level for the two group comparisons. We will recruit 30 participants for each group to allow

for 10% dropouts. The subject enrollment will be split equally between the two sites (15 participants/group per site). For this study, our primary analysis is to use mixed model to test the interaction between group and time, which is expected to be more powerful than the two-sample t-test because all repeated measurements will be considered in modeling, baseline patient characteristics can be controlled statistically, and participants with missing data can be included for analysis.

D. Subject Criteria

a. Inclusion criteria/Exclusion criteria:

i. Inclusion Criteria

- Ages 35–65 years
- BMI between 25-43 $kg \cdot m^2$
- >5% risk for a cardiovascular event in the next 10 years according to the 2013 American College of Cardiology/American Heart Association risk calculator **and/or** 2 out of 5 metabolic syndrome risk factors (Triglycerides ≥ 150 mg/dL; HDL ≤ 40 mg/dL; Glucose ≥ 100mg/dL; Waist Circumference ≥ 102cm for males, 88cm for females; Blood pressure: ≥ 130mmHg systolic and/or 85mmHg diastolic or being treated for hypertension) **and/or** LDL-Cholesterol >120 mg/dl.
- Stable doses of medications for 90 days
- Willing to stop all NSAIDs and aspirin for 7 days prior to muscle biopsy

ii. Exclusion Criteria

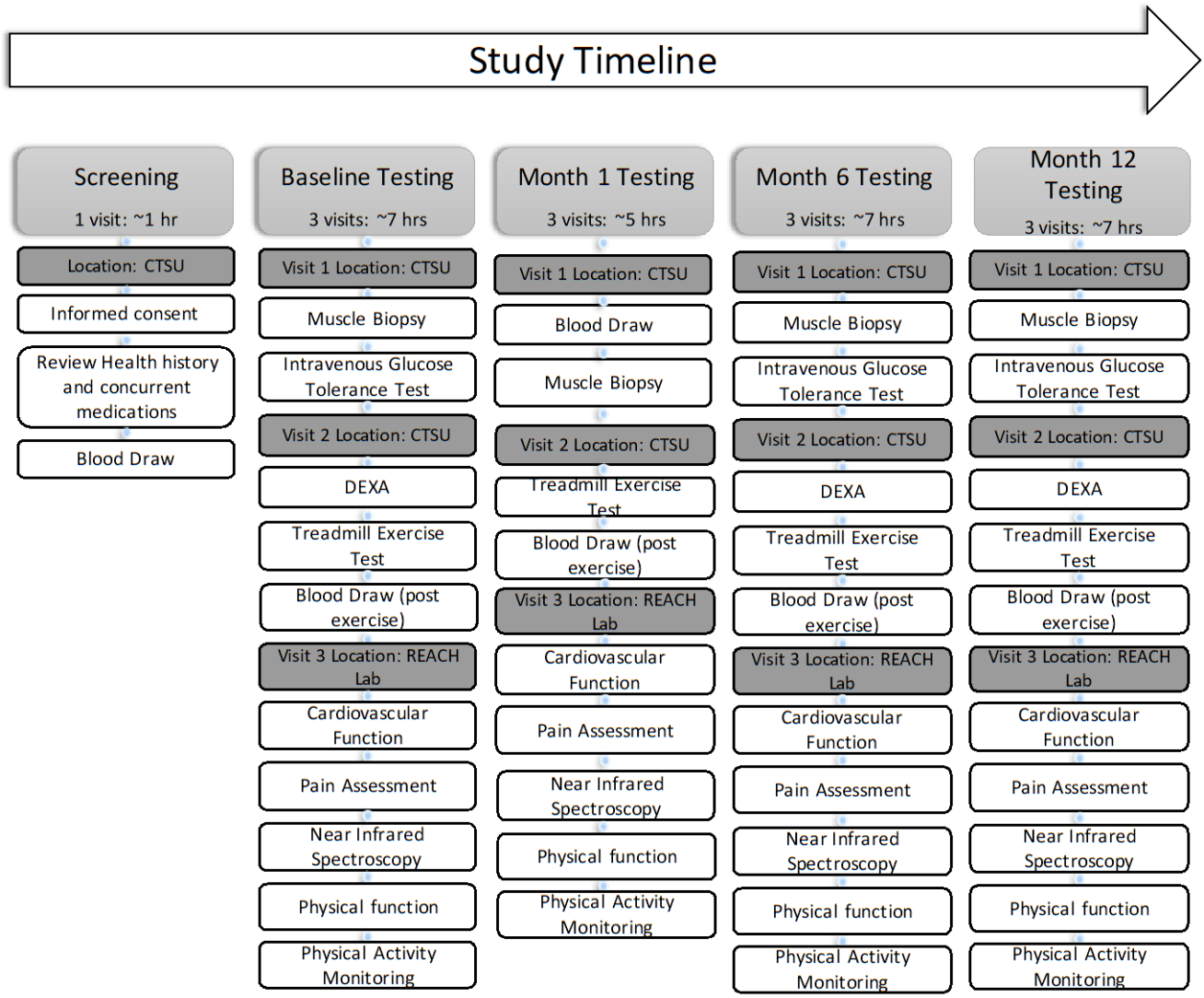
- Smoking
- Previous use of statins
- Use of other medications or supplements that affect lipid profiles or body weight in the last 6 months
  - e.g., fibric acids, bile acid sequestrants, nicotinic acids, fish oil
- Diagnosis of chronic diseases including CVD, other metabolic diseases (e.g., thyroid), current diagnosis and active treatment of cancer, HIV, or acquired immunodeficiency syndrome.
- Diagnosis of type 1 or type 2 diabetes at the time of screening (fasting blood glucose >126mg/dL). If evidence of type 2 diabetes outcome measures is detected during the course of the study (fasting glucose > 126 mg/dl or HbA1c > 6.5%) we will notify the participant to contact their physician.
- History of abnormal bleeding problems
- Currently taking (within the last 10 days) anti-platelet medication (Plavix), Warfarin, and other anti-coagulants (eliquis, pradaxa, and xarelto) medications.
- >2 fold upper normal limit (UNL) for ALT or creatinine
- Women who are pregnant or breastfeeding
- Individuals with polymorphism (SLCO1B1) known to be associated with susceptibility for statin induced myopathies (tested at screening)
- Currently enrolled in another research study

b. Withdrawal/Termination criteria: Participants are free to abstain from taking their statins and leave the study at any time. We will test both muscle enzymes (creatine kinase) and liver enzymes (ALT) to determine if statins are causing either muscle myopathies or liver injury. This testing will occur at baseline and at 1 month into taking

statins. If enzymes are elevated, the subject will be taken out of the study for their own health.

E. Specific methods and techniques

This study will require approximately 13 total testing visits. The total time commitment of study visits is approximately 30 hours over the 12 months of participation.



a. Testing Procedure

i. Telephone screen: A telephone screen will be used to collect health history, concurrent medications, and physical activity level of potential participants prior to in-person screening.

ii. Blood draw: A screening blood draw will be used to determine presence of genetic markers associated with increased risk of myopathy from statin, creatine kinase (CK), kidney function (GFR) and liver enzymes (ALT). CK, GFR and ALT will be used to detect abnormal muscle, kidney or liver function prior to enrollment. A blood draw will be repeated at 1 month to measure for myopathy, and changes in

liver and kidney function from statin use. The study physician will be allowed to request additional lab tests to ensure participant's safety. In some cases, when the participant is not close to any milestone, that will mean an additional visit to obtain the blood draw.

iii. Brief Pain questionnaire: this survey will be used at screening, month 1, 6, and 12 to subjectively assess general pain in participants. This measure will be used to measure if pain levels have changes during the intervention. It will specifically be useful in participants who decide to withdraw.

iv. Intravenous glucose tolerance test (IVGTT): The subject will arrive following an overnight fast (10-12 hrs). Upon arrival, anthropometrics and vital signs will be measured. A catheter will be placed in one arm for blood draws. A fasting blood draw (~14-16mls) will be used to determine blood glucose, insulin, total cholesterol, HDL-C, LDL-C, triglycerides. Creatine kinase (CK) and liver enzymes (ALT) will be tested at 6 and 12 months during IVGTT. CK and ALT will be used to detect abnormal myopathy, and changes in liver function from statin use for participant safety. Following the baseline draw glucose (50%) will be injected at a dose of 0.3 g/kg. First, 10 units of insulin will be pulled into an insulin syringe. Then 90 units of saline to fill the syringe. The mixture with be wasted down to the calculated dose (body mass in kg X 0.025). Then 4 ml of saline will be pulled into a 5ml syringe. The insulin/saline mixture will then be injected into the 5ml syringe. A dose of 0.025 U/kg body mass will be administered at minute 20. These mixing procedures have been safely preformed at the CTSU for a recent study (INFORM; HSC#13453). Serial blood samples (28 total) will be obtained from the other arm at minutes 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180. Plasma will be frozen at -80°C for later determination of glucose and insulin, and insulin sensitivity will be calculated using a minimal model program (77). A total of approximately 622 ml of blood will be collect over the 12 month intervention. After analysis for current project is complete any remaining blood samples will be stored indefinitely for future research. Fasting blood draw will be repeated at 1, 6, and 12 months. The IVGTT will be repeated at 6 and 12 months.

v. Muscle biopsy**:** After an overnight fast, the participant will complete a survey to confirm the absence of bleeding disorders, and avoidance of NSAIDs and aspirins for 7 days prior to biopsy. Participants will then lie on a table in a supine position. A location on the mid-thigh will be identified. The area will be properly shaved and then thoroughly cleaned using sterile techniques (alcohol and iodine). A sterile drape is then placed over the leg with the biopsy site showing through a fenestration in the center of the drape. A numbing agent will be lightly sprayed onto the biopsy location (ethyl chloride) immediately before the skin, adipose, and muscle fascia is anesthetized with lidocaine (1-2% HCL) or diphenhydramine 1% injectable solution for those with 'caine' allergies. After allowing 1-2 minutes for the site to become numb the skin and fascia is incised with a sterile scalpel (#11). The Bergstrom needle (5mm) is then placed through the incision into the vastus lateralis muscle. Suction is applied to the proximal port of the needle, and the tissue is cut rapidly with the internal portion of the needle, and the entire needle

is removed from the incision. Sterile gauze is placed on the incision site and pressure is applied with an ice pack for ~5 minutes. The site is then closed with a steri-strip and band-aid. Sterile gauze is then applied on top of the band-aid and pressure is applied by wrapping coban around the thigh. Approximately 100-200 mg of skeletal muscle will be collected during each biopsy in one pass. In the case where less muscle is harvested per pass, one more additional pass will be taken with the participants consent. The tissue collected is dissected free of connective tissue and separated for mitochondrial function studies or frozen in liquid nitrogen for subsequent biochemical analyses as done previously (13, 61). Dr. Thyfault and his team have performed over 300 skeletal muscle biopsies without incidence since starting his independent lab in 2005. Additionally, Neurologists who study various muscle diseases regularly perform the technique at KUMC. After analysis for current project is complete any remaining muscle samples will be stored indefinitely for future research. Muscle biopsies will be repeated at 1, 6, and 12 months. See appendix for letter from IRB Director (Michele Kennett) at Dr. Thyfault's previous institution (MU) stating his record for conducting muscle biopsies and a letter of support for the procedure from Dr. Jeff Burns, the Director of the CTSU. Biopsies will be taken by Dr. Thyfault or by Dr. Harrison Stierwalt who has been trained during his PhD work at Oregon State and by Dr. Thyfault here at KUMC

vi. Near Infrared Spectroscopy (NIRS)**:** Muscle mitochondrial functional capacity will be measured non-invasively using a NIRS technique developed by Dr. Terence Ryan, a member of the research team at ECU (46). Participants will perform submaximal knee extension exercise to increase muscle oxygen consumption (mVO_2) and the recovery kinetics is determined during a series of repeated arterial occlusions. The mVO_2 data are fit to a mono-exponential function to calculate the rate constant, which is directly related to the mitochondrial respiratory capacity. NIRS will be repeated at 1, 6 and 12 months.

vii. Pain Assessment**:** Pressure pain threshold will be objectively assessed by a computerized algometer at rest and after exercise (73). In addition, we will ask participants to rate their current and usual whole body muscle pain while resting and moving using 0-10 numeric scales (0 = no pain, 10 = most intense pain imaginable) at different time points. We will ask the participants to rate the highest pain felt immediately and for up to 30 minutes after each maximal exercise test, as well as 24 hours later. Pain assessments will be repeated at 1, 6, and 12 months.

viii. Muscular Strength Assessment: Knee isometric strength will be assessed using a Biodex dynamometer. Participants will be seated in a specialized chair and leg will be strapped to the dynamometer arm. Participants will also be secured to the chair to ensure isolation of the knee joint. Trained staff will instruct participants on how to gradually develop force in one second. Following 3 practice trials of submaximal contraction of both flexion and extension, participants will be asked to alternate between maximal flexion and extension with 60 seconds rest between contractions. There will be 3 trials of flexions and extension on both legs. Muscular strength assessments will be repeated at 1, 6, and 12 months

ix. Physical Performance Assessment**:** A series of physical performance measurements will be performed to access balance, gait speed, and strength (74). The physical performance will be repeated at 1, 6, and 12 months.

x. Body Composition**:** Participants will be evaluated with dual energy x-ray absorptiometry (DEXA, Lunar Prodigy, version 11.2068, Madison, WI) to determine fat-free mass, fat mass and percent body fat at. DEXA uses very low X-ray doses (0.02mREM) to detect changes in body composition. DEXA body composition will be repeated at 1, 6, and 12 months.

xi. Exercise Testing**:** Whole body steady state submaximal O_2 consumption will be determined during a standard 6 min treadmill test (2.5mph) to determine VO_2 cost. Participants will then complete a ramped treadmill test (Bruce protocol) to determine cardiorespiratory fitness (VO_2 peak) and peak heart rate as previously described (13). We will also investigate post exercise oxygen consumption and heart rate at 60, 90, 120, and 300 seconds after cessation of the max test to determine if exercise recovery is impacted by statins. Blood will be sampled for measurement of CK 20-30 minutes following the maximal exercise to assess potential muscle damage. Exercise testing will be repeated at 1, 6, and 12 months.

xii. Cardiovascular Function**:** These measures include both heart rate and stroke volume performed under both resting and sub-maximal exercise conditions (performed on separate days) (80). Stroke volume will be estimated by finometer placed on finger (80). Cardiovascular function will be repeated at 6 and 12 months.

xiii. Physical Activity Monitoring: Physical activity levels and sedentary time will be assessed by pedometers and accelerometers for 5 day periods. Physical activity monitoring will be repeated at 1, 6 and 12 months.

xiv. Statin Therapy Intervention: Once participants have successful completed baseline testing, the participant will be randomly assigned to 1 of 3 groups (1:1:1 ratio). Statins will be prescribed as a placebo or at a dosage of 20 or 80 mg/day (atorvastatin; Lipitor) by the study physician at KUMC, Dr. John Miles, MD. Participants and research team will be blinded to the type and dose of statin. Statins will be dispensed by KUMC investigational pharmacy at regular intervals during the participant's enrollment in the study. The study physician will provide care for any problems associated with the statin treatment after un-blinding by Data Safety Monitoring Board, Dr. Megan Baumgardner. All doses fall within the norms of recent recommendations and are given chronically (2).

F. Risk/benefit assessment:

a. Potential risks:

i. Exercise: Some risks are associated with the exercise testing used to assess maximal aerobic capacity. Cardiovascular events during maximal exercise and in recovery are possible but rare in this population (1). However, these risks could be serious. Therefore, initial screening by maximal stress test will be performed in a clinical setting under the direction of a medical monitor (medical monitor only supervises the graded exercise test). Muscle soreness and strain are possible with exercise testing, but these are not a serious risk. The maximal aerobic capacity test represents greater risk of cardiovascular events than the training exercise because of the increased intensity of the exercise. However, events are extremely

rare, and participants will be screened for risk of cardiovascular disease and symptoms. Emergency equipment, including defibrillator, and ambulance plan are available for all participants.

ii. Statins: Statin therapy is associated with muscle issues that range from more minor issues of muscle pain, cramping and fatigue, to myopathies, to the very severe issues of rhabdomyalgia, although this is rare. Statins are the most prescribed drug in the world. Statins are also associated with headaches, nausea, and constipation. Statins are also not well tolerated by athletes suggesting that exercise may worsen the effects of statins on skeletal muscle. Thus, there is the potential that participants in our study may have adverse effects while taking statins. A recent trial conducted by our consultant, Dr. Paul Thompson, utilized 80 mg/day of atorvastatin and found that ~15% of participants experienced pain and weakness (2). atorvastatin 80 mg is an increasingly common dose because three large clinical trials, REVERSAL (atorva 80 v. prava 40, n=502) (3), PROVE IT (atorva 80 v. prava 40, n=4,162) (4), and TNT (atorva 80 v. prava 10, n=10,001) (5) demonstrated reduced atherosclerosis and cardiac events with atorvastatin 80 mg. These studies were done in CAD patients, a group on multiple other medications and likely to be at higher risk for side effects than our healthy participants. Nevertheless, side effects were rare. Liver function test (>3 times upper normal limits [UNL]) and creatinine kinase (CK) elevations (> 10 UNL) occurred in only 1-3.3% of patients treated with atorvastatin 80 mg for up to 24 months, respectively. These side effects should be even more rare given that we will pre-screen and exclude individuals with polymorphisms (GATM and SLC01B1) that increase risk for myopathy. We will test both muscle enzymes (creatine kinase) and liver enzymes (ALT) to determine if statins are causing either muscle myopathies or liver injury. This testing will occur at baseline and at 1 month into taking statins. If enzymes are elevated, the subject will be taken out of the study for their own health. Finally, study physicians at both KUMC who commonly prescribe statins and deal with patients who experience statin induced problems will provide medical coverage for our studies. Participants are free to abstain from taking their statins and leave the study at any time. Importantly, most individuals report that statin induced complications go away very quickly after stopping the therapy. Participants who are discontinued from the study for a medical reason judged to be appropriate by the study doctors will receive the same reimbursement as if they completed the study to avoid the possibility that a subject would "soldier on" simply for the reimbursement.

iii. Muscle Biopsies: Acute issues of muscle tightness, soreness, and bruising can be associated with muscle biopsies. More severe problems include the risk of infection. To avoid these issues the biopsy procedures will be conducted with sterile techniques with medical supervision. Moreover, the participants are given detailed instructions including how to take care of the incision site and who to contact should anything seem abnormal.

iv. IVGTT Catheter Placement and Venipuncture: Their risk is very minimal. However, certain risks including discomfort, blood clot, minor bleeding, bruising, infection,

and redness can occur. Aseptic techniques will be used to minimize such risks. Risks of bruising and minor pain can occur with venous phlebotomy. Venous catheter placements can rarely cause bleeding, bruising, soreness, and infection. Risk of bleeding after removal of the line also is possible. Infusion of glucose or insulin may cause the subject to feel nauseous or flushed. Drinks and snacks will be available for the participants at the completion of the IVGTT should they have low blood sugar or feel nauseous.

v. DEXA: The DEXA scan may make participants slightly uncomfortable because you have to hold very still. This research study involves exposure to radiation equal to the radiation that most Americans receive in about 2 days from background radiation, such as naturally occurring radioactivity in the soil and air. The risk from radiation exposures of this magnitude is too small to be measured directly and is considered to be very low when compared with other everyday risks.

b. Potential Benefits: All participants will gain health information about themselves. At the end of the study, results collected will be shared with the subject in face-to-face meetings. Moreover, although we are studying the impact of statins on muscle health, statins overall do have a strong track record of lowering blood lipids and in most trials lowering cardiovascular risk. Moreover, the health information gained throughout the study will aid the participants in making future medical decisions with their physicians in relation to cardiovascular and diabetes risk.

In summary, the benefits to the participants are substantial, and the information detailing the impact of statins on mitochondrial, metabolic, and cardiovascular function have the promise of helping millions of patients and physicians evaluate the risk to benefit ratio of statin therapy. There are risks associated with the study, but the experience and medical expertise of the research team should keep these at a minimum, and our track record with exercise and diet research indicates that this diligence has been effective.

G. Location where study will be performed: All portions of the study will occur at KUMC.

H. Collaboration: This is a Multi-PI application from Drs. John Thyfault and Darrell Neufer from the University of Kansas Medical Center and East Carolina University, respectively. Both investigators have been conducting research on statins for several years. The project was born out of a common desire to combine their individual expertise to investigate the mechanisms underlying skeletal muscle complications associated with statin therapy in a repeated measures, longitudinal design. Due to the scope of the design (i.e., number of participants, duration) and complexities of the primary outcome measures, it was felt a multi-PI, multi-institutional approach represented the most efficient and statistically valid means of completing the project and thereby making truly sustained impact of the field. Each investigator has trained at/frequently visited the other institute and is quite familiar with the research environments. Standard operating procedures will be developed for all common testing and primary outcome measurements, and each investigator and their key personnel will visit the other institute prior to commencing the study to conduct trial runs to insure continuity. The PIs will share responsibility for fiscal and research management. Using the multi-PI approach with two investigators with translational research experience and

complimentary analytical expertise dramatically increases the power of the study and the likelihood of successfully completing the proposed aims.

I. Single IRB Review for a Multi-site study: NA

J. Personnel who will conduct the study, including:

1. Indicate, by title, who will be present during study procedure(s): PI, Study Coordinator
2. Primary responsibility for the following activities, for example:
    a. Determining eligibility: PI, Study Coordinator
    b. Obtaining informed consent: PI, Study Coordinator
    c. Providing on-going information to the study sponsor and the IRB: PI, Study Coordinator
    d. Maintaining participant's research records: PI, Study Coordinator
    e. Completing physical examination: PI, CTSU Nurse
    f. Taking vital signs, height, weight: CTSU Nurses
    g. Drawing / collecting laboratory specimens: CTSU Nurses
    h. Performing / conducting tests, procedures, interventions, questionnaires: PI, Study Coordinator
    i. Completing study data forms: Study Coordinator
    j. Managing study database: PI

K. Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

a. Key personnel: John P. Thyfault, PhD, Sandy Billinger, PhD, Darrell Neufer, PhD, John Miles, MD, study physician at KUMC and Bruce Ferguson, MD, study physician at ECU.

b. Plan for safety monitoring and review: Dr. Megan Baumgardner will serve as the Chair of the Data Safety Monitoring Board and will convene every 6 months with the other members, Drs. Nicol and LeMaster to make decisions on safety of the participants. Enrollment data will also be reviewed twice a year by the DSMB. In addition, data will be reviewed on a yearly basis with the biostatistician, Dr. He to determine if the studies should be shortened do to either no measured effect or due to effects that are greater than what power calculations indicated. If problems occur with participants, blinded study staff will alert Dr. Baumgardner. Dr. Baumgardner will have access to dose and type of statin and will consult the study physicians/cardiologist to make a decision on whether the subject should be removed from the study for safety concerns. The PI and other key personnel will be examining research data on a subject to subject basis to ensure it is accurate and to address any potential problems that may arise with the protocols. In addition, Drs. Neufer and Thyfault will discuss data at least once per month through in person meetings or by phone to ensure that data collection is occurring in a consistent manner at both locations.

c. Plan for adverse event reporting: Adverse events will be defined as any untoward medical occurrence in study participants or others immediately involved in the

performance of the protocol, which does not necessarily have a causal relationship with the study treatment, but results in a change in intervention, daily function, hospitalization or rated category 3 or above using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v3.0. Expected events such as slight muscle soreness consistent with statin therapy, or those consistent with a participant's prior medical history not sufficient to alter the intervention will not be considered an AE. Staff involved in performance of the protocol (e.g. coordinators) will continually monitor participants for adverse events throughout the intervention, and study staff will assess adverse events at every testing visit as well as during regularly scheduled telephone assessments with all randomized participants.

If study staff, tester, or participant reports adverse events or complaints, relevant information will be collected and documented. The participant will be evaluated by the unblinded investigator identified in the protocol. The investigator will determine the severity (according to the CTCAE) and relatedness of the AE to the intervention. The investigator will identify adverse events of clinical concern, those that may require further workup, or suggest that additional participation in the intervention might be a safety risk. These AEs will first be discussed with the participant and then communicated to the study staff or providers with participant's consent and as appropriate. Serious adverse events directly related to the intervention will be reported immediately to the IRB. Any adverse event rate over 30% in 12 months will be reported to the NIH.

III. Subject Participation

A. Recruitment:

a. Participants will be recruited from the KUMC campus, from hospitals and clinics associated with KUMC and metro-Kansas City, Kansas and Missouri areas. Study team will also use BPA system through Epic to recruit participants. Recruiting will occur through physicians and clinics and through advertisements in emails, flyers, newspaper ads, radio spots, etc. Interested participants will call or email and undergo initial phone screening, conducted by trained study staff, to see if they qualify. Should they meet the initial qualifications, they will come to the lab for an informed consent meeting with the PI, research coordinator, or other study staff. They will then be provided with an informed consent that is fully approved by the IRB at KUMC to review prior to the in-person meeting. Participants who are interested in taking part in the study will be asked to sign the informed consent in the presence of a witness. Participants will have adequate opportunity to review the informed consent and to ask any questions they may have about the research protocol, compensation, risks, and benefits of taking part in the study.

b. The targeted/ planned distribution of participants by sex/gender and racial/ethnic groups for the proposed study or protocol will follow the local demographics. The Kansas City area has a population that is comprised of 13 % African American, 2% Asian, 5% Hispanic, and 80% Caucasian. It is anticipated that the study population at KUMC will have much the same racial and ethnic make-up. Statistical comparisons among minorities or between minority and Caucasian groups are not aims of the current study. We fully intend to enroll every eligible minority individual in these studies. Ample pools of all targeted populations exist.

c. Selection criteria: It is anticipated that women will comprise approximately 50% of the study participants. It is also anticipated that gender will not be a significant factor in the response to exercise training or statin therapy, and hence, it is anticipated that the data from both genders will be compiled and analyzed as a group.

d. Exclusions: No minority groups will be excluded.

e. Outreach: The subject pool will be limited to the greater Kansas City (MO and KS) area. Subject will need to make regular visits to the laboratories, and thus, participants will only be recruited who are within the county region and can regularly make appointments.

B. Screening Interview/questionnaire: NA

C. Informed consent process and timing of obtaining of consent: Should the participant meet the initial qualifications, they will come to the lab for an informed consent meeting with the PI, research coordinator, or other study staff. They will be provided with an informed consent that is fully approved by the IRB at KUMC to review prior to the in-person meeting. Participants who are interested in taking part in the study will be asked to sign the informed consent in the presence of a witness. Participants will have adequate opportunity to review the informed consent and to ask any questions they may have about the research protocol, compensation, risks, and benefits of taking part in the study.

D. Alternatives to Participation: NA

E. Costs to Participants: There is no cost to participants for participation in this study.

F. How new information will be conveyed to the study subject and how it will be documented: New information about risks associated with involvement in the study will be disseminated but no other information will be disclosed.

G. Payment, including a prorated plan for payment: Participants will receive a one-time compensation of $600 for completing all study visits. If participation ends early, participants will receive $75 for each study visit excluding screening. They will be given a ClinCard, which works like a debit card. After completion of the study, payment will be added to the card by computer. The money will be available within 1 business day. Participants can use the ClinCard at an ATM or at a store. Study staff will collect their name, address, and social security number to allow them to set the participant up in the ClinCard system through the KUMC Research Institute. Study payments are taxable income. A form 1099 will be sent to the subject and the Internal Revenue Service if your payments are $600 or more in a calendar year. The participants' personal information will be kept on a secure computer. It will be removed from the computer after the study is over and the money on the card has been used. The information will not be shared with other businesses. It will be kept confidential.

H. Payment for a research-related injury: We will include the following text in the consent form: "All forms of medical findings, whether routine or experimental, involve some risk of

injury. In spite of all safety measures, you might develop medical problems from participating in this study. You must report any suspected illness or injury to the study coordinator immediately. If such problems occur, you will be provided with emergency medical treatment and the investigator will assist you in getting proper follow-up medical treatment. Neither the investigator nor the sponsor will provide compensation for research-related injuries. Payment of lost wages, disability or discomfort is not available. You do not give up any of your rights by signing this form."

IV. Data Collection and Protection

A. Data Management and Security: All identifiable files, data, and tissue will be coded and stored in secure locations. These data include: study progression, subject numbers, percent completion, data quality, subject retention, adverse events, etc.
The PIs at both KUMC and ECU will be responsible for the quality of the data and will supervise the data acquisition with the help of study coordinators. All data will be coded for confidentiality, and only the PI, study coordinator, and study statistician will have access to the code. The results of each test on each subject will be screened by the study coordinator prior to entering on the spreadsheet. This is standard practice in our laboratories. Hard copies are kept in dedicated locked cabinets. Specific coded subject files will be sent to the study statistician for statistical analyses. These analyses will be sent to the PI and study coordinator for dissemination to appropriate co-Is. At the end of participation in the study, each subject will receive a personal data summary of their test results. The study coordinator or a study investigator will discuss the results with the subject.
After 1 year, the PI's including the biostatistician, Dr. Jianghua He will be un-blinded but the study staff will remain blinded, so that the important research findings can begin to be delineated. This will also allow for Dr. He to being to examine if the trial is finding more or less significant effects for outcome measures.

B. Sample / Specimen Collection: All participants will be assigned a data code. Blood and tissue samples will be labeled with the participant's data code and stored in a locked freezer. Blood and tissue samples will be stored indefinitely following completion of current project for future research.

C. Tissue Banking Considerations: Following completion of the current project, remaining blood and tissue samples will remain in locked freezer indefinitely. Samples will only be used by investigators of the current project, both KUMC and ECU, when a new biomarkers or techniques of interest emerge.

D. Procedures to protect subject confidentiality: Risks to confidentiality are reduced by assigning all participants to a data code. Folders are stored in locked file cabinets, and only the PI and study coordinator have access to the locked files. Individual names or initials are not used in any discussions or publications of the data.

E. Quality Assurance / Monitoring: Data will be reviewed twice a year by the Board Chair. In addition, data will be reviewed on a yearly basis with the biostatistician, Dr. He to determine if the studies should be shortened do to either no measured effect or due to effects that are greater than what power calculations indicated. The PI and other key

personnel will be examining research data on a subject to subject basis to ensure it is accurate and to address any potential problems that may arise with the protocols. In addition, Drs. Neufer and Thyfault will discuss data at least once per month through in person meetings or by phone to ensure that data collection is occurring in a consistent manner at both locations.

V. Data Analysis and Reporting

A. Statistical and Data Analysis: This project is a two-factor study. One factor is treatment group (between participants) and a second factor is time of data collection (0, 1, 6, and 12 months) (within subject). The data analysis will be carried out for the primary outcome variable of mitochondrial respiration in addition to other outcome variables (VO_2peak and insulin sensitivity, etc.). For each measure, its baseline measure and age will be added as covariates to adjust for the individual difference at baseline. Two-way interaction between treatment and time will be added in the model for testing if the outcome changes differently between groups over time. Additional variables, such as demographics, BMI, physical activity, etc. may also be included as covariates. If the test of trend difference is significant, then the comparison at each time point will also be considered to identify when the difference between groups reaches the maximum. Least Square Means will be used for the aforementioned comparisons. The statistical analysis will be performed with mixed procedure in STATA 13.1 (StataCorp LP, College Station, TX). Mixed model does not require complete data for a longitudinal study. Residual analyses will be conducted and efforts to address those will be made, including appropriate transformations.

B. Expected Outcomes: Atorvastatin therapy will induce a progressive decline in skeletal muscle mitochondrial respiratory capacity that will be evident both in vivo (i.e., whole muscle NIRS) and in situ (i.e., respirometry in permeabilized fiber bundles). Based on the rationale outlined above and our preliminary data, we anticipate mitochondrial function will decline in a dose x time-dependent manner, occurring sooner in the high- verses low- dose treatment group. We further predict that the effect will be inversely related to the initial mitochondrial respiratory capacity of the skeletal muscle. Loss of mitochondrial functional capacity/content is predicted to, in turn, compromise the ability to maintain cellular redox homeostasis. Based on our previous findings (61), a shift to a more oxidized cellular redox state in skeletal muscle is expected to negatively impact insulin action. We also anticipate mitochondrial Ca2+ retention capacity will be reduced by statin therapy, providing a potential mechanism for the loss of mitochondrial content over time. If this occurs, follow up measures of genes and proteins controlling Ca2+ retention capacity (mitochondrial calcium uniporter and antiporter), and apoptosis and mitophagy (Bcl-2 (anti-apoptotic)/Bax (pro-apoptotic) will be performed to determine if these pathways are altered by low or high dose statins. Consistent with the reductions in mitochondrial function, we expect whole body insulin sensitivity and cardiorespiratory fitness (VO2 max) to also be reduced in a dose and time dependent manner (i.e., downstream of declines in mitochondrial function) in response to statin therapy. Of particular interest will be to determine whether reductions in mitochondrial respiratory capacity are associated with lower pain threshold and higher CK levels at rest and/or in response to exercise testing, and whether such changes are associated with compromised indices of cardiovascular function (increased heart rate and/or

reduced stroke volume), potentially occurring secondary to mitochondrial dysfunction in skeletal muscle or in another cell type like endothelial cells. Most of the data, since it is collected in real-time, will be analyzed as the study progresses allowing us to stop the study early should statistical differences in the primary outcome measures be detected prior to completion.

C. Study results to participants: At the end of participation in the study, each subject will receive a personal data summary of their test results. The study coordinator or a study investigator will discuss the results with the subject. Results to be shared include: Body composition, exercise testing results, lipid profile, liver function (ALT), and glucose and insulin results from IVGTT, and summary of physical activity levels.

D. Publication Plan: We will publish all data derived from this award in a timely fashion and to make those data freely available to the general research community whenever possible. All genomic data will be made publicly available through NCBI's Gene Expression Omnibus (GEO), and all publications that result from this work will be deposited in PubMed Central (PMC).

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