University of Kansas Medical Center
RESEARCH PROTOCOL INVOLVING HUMAN SUBJECTS

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Study Title: Impact of statin therapy on adaptations to aerobic exercise

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I. Purpose, Background and Rationale

A. Abstract

More than 40 million Americans are currently taking 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 and blunt adaptations to exercise. The overall aim of the study is to examine whether statin treatments differentially blunt the mitochondrial, metabolic, and cardiorespiratory adaptations to aerobic exercise training. Our hypothesis is statin therapy will impair exercise induced adaptations in skeletal muscle mitochondria content and function (respiration and transcriptional responses), cardiovascular responses, and systemic insulin sensitivity.

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.

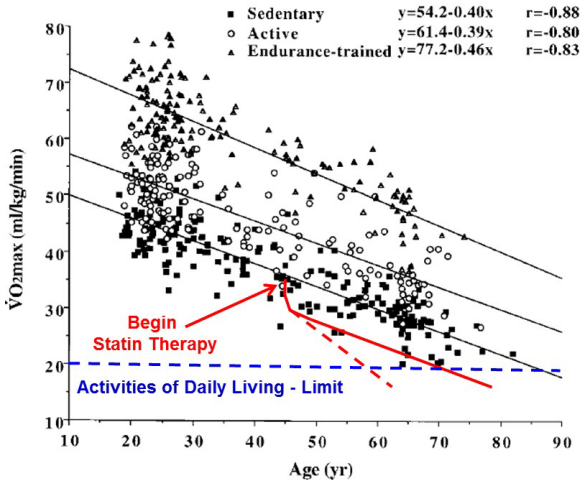

Fig 1. Relationship between maximal oxygen uptake and age in sedentary, active and endurance trained subjects. The figure is adapted from Wilson and Tanaka (1) and depicts the potential impact of statin therapy to shift (solid red line) or accelerate (dotted red line) the loss of aerobic capacity relative 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 are a first line of treatment for patients at risk for the metabolic syndrome and CVD because they lower lipids (LDL-C and TG) and reduce CVD. Although, statins undoubtedly reduce CVD, they have been shown to increase both insulin resistance (8, 9, 20, 21) and rates of type 2 diabetes development in large cohort studies (6, 7, 19). Importantly, the mechanism(s) linking statins to insulin resistance and type 2 diabetes remain unknown. Statin induced mitochondrial dysfunction in skeletal muscle is one of the suggested mechanism(s) for statin induced insulin resistance, but this has not been directly tested.

Although statins provide a powerful 20-40% reduction in relative risk for CVD (19, 85-87), there is still room for improvement through the use of potent lifestyle therapies like exercise training. At risk patients are commonly encouraged to start a regular aerobic exercise program, that can also lower LDL-C and TG, and unlike statins, is a proven efficacious treatment to improve insulin sensitivity and protect against type 2 diabetes (88, 89). The ability of exercise to increase skeletal muscle mitochondrial content and function is believed to play an important role in improving skeletal muscle insulin sensitivity. Exercise also increases cardiorespiratory fitness (VO_{2peak}), which is a critical adaptation because low fitness is a powerful independent risk factor for CVD, type 2 diabetes, and overall mortality (26, 27, 90-92). In fact, mid-life fitness is an excellent prognosticator of susceptibility for chronic disease conditions later in life (93). A recent study implicated statin therapy in reducing physical activity levels by a significant amount in free living individuals (94). Importantly, another trial found that alterations in physical activity (measured by pedometers) significantly impacted CVD risk in at risk patients regardless of pharmacotherapy treatment suggesting that increasing or maintaining physical activity throughout the lifespan is paramount for CVD protection (95). In summary, these findings suggest that the most complete treatment for patients at risk for CVD would combine both statin and exercise therapy in addition to maintenance of moderate to high daily physical activity.

Table 1	Exercise Only (n=19)			Statins + Exercise (n=18)		
	Pre	Post	%Δ	Pre	Post	%Δ
VO_{2peak} (ml/kg/min)	26.9±1.9	29.5±2.3	↑9.5%	26.1±1.4	26.5±1.3	↑2
Citrate Syn (nmol/min/ug)	100.4±6.5	113.6±4.3	↑13%	111.0±5	106.0±5.3	↓-4.5%

Although many clinical reviews propose harmful interactions with exercise and statins, until a recent report from our lab, there were no carefully controlled studies. We published a trial comparing the effects of 12 weeks of exercise vs. exercise + statin therapy (40 mg/day simvastatin) in previously sedentary obese participants with 2/5 metabolic syndrome risk factors (13). Our results showed that statins blocked both exercise induced increases in whole body cardiorespiratory fitness and increases in skeletal muscle mitochondrial content. As shown in Table 1, the exercise only group experienced a 9% increase in cardiorespiratory fitness (VO2peak) and a 13% increase in mitochondrial content (measured by muscle citrate synthase activity), while the statin plus exercise group had 1%, and -4% changes, respectively. We also now have preliminary data in a subset of subjects (n=15) that centrally mediated cardiovascular effects were blunted by statin therapy. Exercise training lowered resting heart rate as expected in the exercise only group (Pre: 67±1.6 vs. Post: 64±1.7; $P<0.05$), but did not lower heart rate in the exercise + statin group (Pre: 64±2.0 vs. Post: 65±2.5). Importantly, statin induced blunting

of exercise adaptations occurred uniformly in all subjects, and did not just manifest in those with statin induced myopathies (no myopathies reported).
We posit that statin induced impairments in mitochondrial function play a primary role in mitigating exercise training responses; however, this hypothesis needs further testing. The next step is to determine if statins modify mitochondrial adaptations including respiratory kinetics, H202 emission, and transcriptional factors that control mitochondrial biogenesis, providing greater mechanistic insight of the potential negative interaction between exercise and statin therapy. Another factor that needs to be explored is if a lower dose of statin therapy will not block or mitigate exercise adaptations. Physicians reportedly lower the dose of statins for individuals who complain of problems with exercise but this approach has not been experimentally tested. In summary, the goals this project are to build upon our previously published data to determine if atorvastatin, now the most commonly prescribed statin, impairs exercise induced adaptations in skeletal muscle mitochondria content and function (respiration and transcriptional responses), cardiovascular responses, and systemic insulin sensitivity. An additional goal is to determine if low dose atorvastatin (20 mg/day) does not block these exercise training adaptations, an outcome that would provide physicians a new treatment option for patients who want to exercise and/or increase daily physical activity but also require statin therapy.

II. Research Plan and Design

A. Study Objectives: The overall aim of the study is to determine whether low or high intensity statin treatments differentially blunt the mitochondrial, metabolic, and cardiorespiratory adaptations to aerobic exercise training.

a. Primary Objective:

i. Changes in Cardiorespiratory fitness (VO2peak): Subjects will be tested for VO2peak at baseline and post training using a Bruce protocol on a treadmill as previously performed (13, 97). Relative (ml/kg-1/min-1) and absolute (L/min-1) VO2peak and treadmill time to exhaustion will be used as determinants of fitness while additional measures of respiratory quotient (RQ) and heart rate at each stage of the GXT, and heart rate recovery and excess post oxygen consumption following the test will also be measured as determinants of exercise training adaptations. Blood CK levels will be assessed 30 minutes following the cessation of exercise and also 24 hour later. In addition, sub-maximal exercise (60% HRR) measures of heart rate, RQ, and O2 consumption will also be determined during exercise training sessions occurring during the first week and the last week of training.

b. Secondary Objectives:

i. Mitochondrial Responses in Skeletal Muscle: 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, 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. An in-vivo assessment of mitochondrial function using NIRS will determine muscle oxygen consumption (mVO2) and the recovery kinetics will be determined during a series of repeated arterial occlusions following maximal knee extension. The mVO2 data are fit to a mono-exponential function to calculate the rate constant, which is directly related to the mitochondrial respiratory capacity. (46). The protein and mRNA for key transcriptional factors controlling mitochondrial biogenesis and function will also be measured (PGC-1α, NRF-1, NRF-2, mTFA) in all subjects as done previously (98, 99). In addition, muscle will be frozen in OCT to examine if there are altered fiber type changes, and or other important markers including apoptosis (TUNEL staining).

ii. Body Composition, Physical Function, Pain Assessments, and Physical Activity:. DEXA will measure bone mineral content (BMC), bone mineral density (BMD) and body composition (fat mass, fat-free mass, and percent body fat). 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. (BodyMedia Sensewear).

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

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

v. Cardiovascular Function: Heart rate ECG will be measured under resting and maximal exercise conditions.

B. Study Type and Design: This study is a longitudinal, repeated measures, double-blinded design with subjects randomly assigned to placebo, low (20 mg/d) or high (80 mg/d) atorvastatin therapy during a 12-week aerobic exercise training protocol. We expect to screen approximately 150 participants to enroll (randomize) a maximum of 80 participants. The overall goal is to have 60 participants complete all study procedures (20 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 the study is powered on the powered on the basis of detecting a difference in VO2peak between the EX+Plac group and the EX+Atorva80 group as well as between the EX+Atorva20 group and the EX+Atorva80 group at 12 weeks post-treatment. In our previously published study(13) (Table 2) the EX only group had a mean 2.6 ml/kg/min increase in VO2peak following 12 weeks of training while the EX+Statin group only had a 0.4 ml/kg/min increase in VO2peak. The estimated effect size of between group differences is 0.9 (Cohen's d). The increase of VO2peak in the EX+Atorva20 group is expected

to be similar to that of EX+Plac group. A sample size of 35 per group provides 80% of power of detecting an effect size of 0.75 for a two-sided, two-sample t-test at 0.025 levels. Here, we use 0.025 for each group comparison to maintain the overall type I error rate at 0.05 level using Bonferroni correction. Assuming variances similar to what was observed in the pilot study, this study is powered to detect a difference of 1.53 ml/kg/min VO2peak between groups. These power calculations led to a desired final sample size of 105 (35/group). We plan an enrollment of 120 (40/group) subjects to account for 10% dropout rate. The subject enrollment will be split equally between the two sites. As an exploratory analysis, the mean difference of VO2peak (EX+ Atorva20- EX+ placebo) and its 95% confidence interval (CI) will be estimated. If the lower bound of the 95% confidence interval is greater than -0.8 ml/kg/min, EX+ Atorva20 will be considered non-inferior to EX+ placebo.

D. Subject Criteria

a. Inclusion criteria/Exclusion criteria:

i. Inclusion Criteria

- Ages 35–60 years
- BMI between 30-39 $kg \cdot m^2$
- Sedentary (less than 30 min of physical activity/week during last 6 months)
- Weight stable (no more than 5% change in body weight the previous 3 months)
- >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).
- Stable doses of medications for 90 days
- Willing to stop all NSAIDs and aspirin for 7 days prior to muscle biopsy
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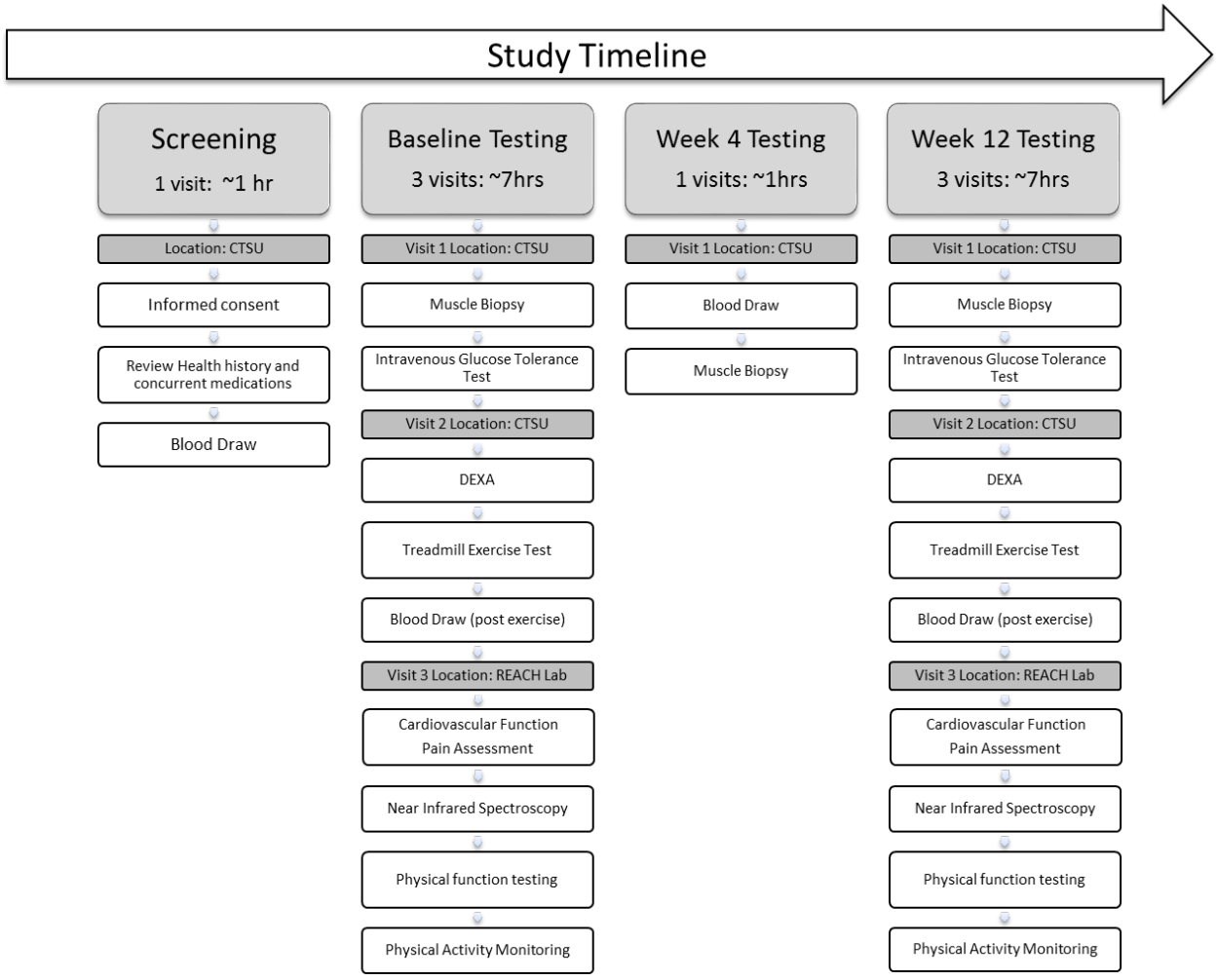
ii. Exclusion Criteria

- 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, diabetes, other metabolic diseases (e.g., thyroid), cancer, HIV, or acquired immunodeficiency syndrome
- 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 polymorphisms (SLCO1B1) known to be associated with susceptibility for statin induced myopathies (tested at screening)
- Currently enrolled in another research study

b. Withdrawal/Termination criteria: Subjects 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 study visits plus exercising 5 times per week. The total time commitment of study visits is approximately 30 hours over the 12 weeks of study participation.



a. Testing Procedure

i. <u>Telephone screen:</u> 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.

iii. 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. Insulin will be mixed with the participant's blood for injection. Blood (5ml) and insulin (1.25 ml) will be injected into a 250 ml bag of sterile saline and mixed thoroughly. 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 (25 total) will be obtained from the other arm at minutes 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 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 , 4 and 12 weeks. The IVGTT will be repeated at week12.

iv. 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). 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). After analysis for current project is complete any remaining muscle samples will be stored indefinitely for future research. Muscle biopsies will be repeated at 4 weeks and 12 weeks. Dr. Thyfault has performed over 300 skeletal muscle biopsies without incidence since starting his independent lab in 2005.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. Additionally, Neurologists who study various muscle diseases regularly perform the technique at KUMC. Muscle biopsies will be repeated at 4 and 12 weeks.

v. 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 week 12.

vi. 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 week 12.

vii. 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

viii. 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 week 12.

ix. 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 week 12.

x. Exercise Testing**:** Whole body steady state submaximal O_2 consumption will be determined during a standard 6 min treadmill test (2.5 mph) to determine VO_2 cost. Subjects 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 week 12.

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

xii. Physical Activity Monitoring: Physical activity levels and sedentary time will be assessed by pedometers and accelerometers (BodyMedia Sensewear) (75, 76) for 5 day periods. Physical activity monitoring will be repeated at week 12.

xiii. Stain 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. Subjects and research team will be blinded to the type and dose of statin. Statins will be dispensed by the 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 the chair of the Data Safety Monitoring Board, Dr. Russell Swerdlow. All doses fall within the norms of recent recommendations and are given chronically (2)

xiv. Aerobic Exercise Intervention: Exercise training will be performed as done previously (13) and will consist of brisk walking and/or slow jogging on a treadmill 5 d each week at approximately 65-75% of each subject's VO2peak, 45 min/session, at an energy expenditure of 1800-2000 kcal/wk, for 12 weeks. The exercise training will follow a three-stage progression: 1. wk 1=30 min, 3 d/wk, 60% VO2max; 2. wk 2=30 min, 5 d/wk, 60% VO2max; and 3. wk 3-12=45 min, 5 d/wk, 60-75% VO2peak. Exercise intensity for each session will be monitored using heart rate monitors every 5 minutes and at least four exercise sessions per week will be supervised within KUMC exercise facilities.

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 and training, 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 subjects will be screened for risk of cardiovascular disease and symptoms. Emergency equipment, including defibrillator, and ambulance plan are available for all subjects. Exercise training will be monitored by trained study staff to reduce risk.

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 subjects. 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 KUMC who commonly prescribe statins and deal with patients who experience statin induced problems will provide medical coverage for our studies. Subjects 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. Subjects 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 are conducted with sterile techniques under medical supervision. Moreover, the subjects 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 subjects 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 approximately the level of 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 subjects 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 subjects in making future medical decisions with their physicians in relation to cardiovascular and diabetes risk. The aerobic exercise intervention will represent a stimulus for the beginning of a permanent improvement in lifestyle and a dramatic reduction in risk of cardiovascular disease.

In summary, the benefits to the subjects 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 subjects, 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.**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 study intervention will be reported immediately to the IRB. Any adverse event rate over 30% in 12 mo will be reported to the NIH.

III. Subject Participation

A. Recruitment:

a. Subjects will be recruited from the KUMC campus, from hospitals and clinics associated with KUMC and metro-Kansas City, Kansas and Missouri areas. Recruiting will occur through physicians and clinics and through advertisements in emails, flyers, newspaper

ads, radio spots, etc. Interested subjects 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. Subjects 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 subjects 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: Only premenopausal women will be included in this study because of the possible complications that hormone replacement therapy may have upon findings. It is anticipated that women will comprise approximately 50% of the study subjects. 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, subjects 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. Subjects 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 Subjects: There is no cost to subjects 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 $400 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 subjects' 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. Coded data 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 subjects 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 subjects 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 subjects) 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 cardiorespiratory fitness, in addition to other outcome variables (muscle mitochondrial change, 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: The EX+Plac group and EX+Atorva20 groups will have a significant increase in VO2peak, stroke volume, and a lowering of resting heart rate, common responses to exercise training. Measures of mitochondrial content (citrate synthase) and function (in-vivo NIRS, and ex-vivo respiration) will improve following the intervention, and will be preceded by increases in transcription factors (PGC-1α, NRF-1, NRF-2, mTFA) known to control mitochondrial biogenesis measured at 1 month. Insulin sensitivity will also improve in the EX+Plac and Ex+Atorva20 groups. In contrast, the EX+Atorva80 group will display diminished mitochondrial and cardiorespiratory adaptations because of compromised respiratory capacity and disturbances in redox balance in skeletal muscle. This may occur due to a blunted exercise induced increase in PGC-1α which is known to up regulate anti-ROS defense enzymes (101). Other cardiovascular adaptations may not improve in the EX+Atorva80 group, which could be due to impaired mitochondria oxygen consumption. We expect measures of pain and function to be triggered by the combination of exercise and Atorva80 treatment. These findings would indicate that higher dose atorvastatin impair exercise adaptations and that physicians should consider prescribing lower dose atorvastatin to patients who plan to utilize exercise training to lower cardiovascular risk. If these findings occur we will have remaining tissues to examine further mechanisms including if these findings are associated with reduced cellular ubiquinone levels, and altered balance between pathways controlling autophagy and apoptosis (Bcl-2 and Bax). These can be further supported by immunoflourescent examination of muscle fiber type changes and quantification of apoptosis by TUNEL staining. Given that a rodent study showed atorvastatin therapy blocked exercise induced increases in Glut4 mRNA and glycogen content (102), and that mitochondrial adaptations are critical for improved insulin sensitivity, we expect the EX+Atorva group will also display impaired improvements in insulin sensitivity.

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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