

UCSD Human Research Protections Program
New Biomedical Application
RESEARCH PLAN INSTRUCTIONS

These are instructions are for completing the Research Plan that is available in MS Word format from the [HRPP website](#).

The headings on this set of instructions correspond to the headings of the Research Plan.

General Instructions: Enter a response in for all topic headings.

Enter "Not Applicable" rather than leaving an item blank if the item does not apply to this project.

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1. PROJECT TITLE

Effects of epicatechin on patients with statin-induced mitochondrial dysfunction and impaired exercise capacity

2. PRINCIPAL INVESTIGATOR

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3. FACILITIES

UCSD Altman Clinical and Translational Research Institution (ACTRI)

UCSD School of Medicine

4. ESTIMATED DURATION OF THE STUDY

6 years

5. LAY LANGUAGE SUMMARY OR SYNOPSIS (no more than one paragraph)

Statins are an iconic drug class that has improved mortality in patients with cardiovascular disease. However, up to 20% of patients who must be on this important drug do not take it due to skeletal (SkM) associated side effects that range from muscle cramps to fatigue. We have previously demonstrated that 3 months of treatment of heart failure and type 2 diabetes patients with Epi-rich cocoa recovers mitochondrial structure (cristae) on electron microscopy as well as the levels of proteins associated with mitochondrial biogenesis (MiB). Other recent epidemiological studies have demonstrated that like statins, cocoa and/or Epi consumption has pleiotropic beneficial effects including lowering blood pressure, decreasing inflammatory mediators and platelet aggregation, and protection from ischemic cardiac injury. Thus, a combination of Epi and statins may even be synergistic in improving cardiovascular (CV) mortality. We hypothesize that Epi can block statin related mitochondrial dysfunction and associated decreases in exercise tolerance leading to improved patient compliance.

6. SPECIFIC AIMS

The major objective of this proposal is to assess changes in triglyceride levels and gain additional mechanistic insight into statin associated perturbations in mitochondrial function and impaired exercise capacity and to perform a preliminary evaluation of the capacity of the cacao flavanol (-)-epicatechin (Epi) to limit and/or reverse these changes.

The mechanisms of statin side-effects are poorly understood and thought to be partly due to impaired mitochondrial structure/function. Recent studies have also shown that patients taking statins have decreased exercise capacity. The goal of this study is to overcome statin associated side effects by giving patients on statins a natural supplement derived from dark chocolate (epicatechin) that based on our prior studies can improve mitochondrial function and potentially counteract the side effects of statins.

We will conduct a double-blind placebo controlled clinical trial in which patients who will benefit from statin therapy will be randomized to either the statin only group or the statin+epicatechin group. We will compare exercise capacity and assess mitochondrial function in both groups. We will assess if epicatechin can counteract the side effects of statins and increase patient compliance. This work will help translate our research on beneficial effects of epicatechin into a clinically relevant patient population.

The overall objective of our proposal is to gain additional mechanistic insight into how statins cause impairments in mitochondrial function leading to impaired exercise capacity and perform an evaluation on the capacity of epicatechin to limit and/or reverse these changes. Strategies are needed to increase patient compliance with statins. We believe this work will provide significant understanding of mechanisms that underlie statin related mitochondrial dysfunction and impaired skeletal muscle performance and provide a potential avenue for an effective and safe treatment with epicatechin with the ultimate goal of increasing patient compliance and reducing cardiovascular mortality.

We will test this hypothesis by pursuing the following aims:

Aim 1: Assess the added effects of Epi in reducing triglyceride levels, and in improving/maintaining VO2 max in patients with metabolic syndrome.

Hypothesis: Patients in the statin+epi group will have significantly lower triglyceride levels compared to those in statin only group. We propose to test this hypothesis in a double-blind placebo controlled clinical trial in which patients with metabolic syndrome initiating statin therapy for primary prevention will be followed for 12 weeks with exercise testing and optional SkM biopsies before and after treatment. We will assess:

- *Changes in lipid levels*
- *Functional Capacity (VO2 max, exercise capacity)*
- *Mitochondrial Function (Oxygen consumption rates assessed via Seahorse analyzer) from skeletal muscle biopsy specimens.*

Aim 2: Characterize statin associated changes in functional status in patients with metabolic syndrome starting statin therapy. *Hypothesis: Statin therapy will trigger decreases in mitochondrial structure/function parameters leading to decreases in VO2 max.*

Exploratory Aim 1: Assess the added effects of Epi in reducing LDL levels. *Hypothesis: Patients in the statin+epi group will have significantly lower LDL levels compared to those in statin only group.*

Exploratory Aim 2: Interrogate for possible blood-based biomarkers that correlate with changes in muscle biopsy.

We will be performing the abscorbyl radical assay (which is indicator of oxidative stress) from plasma and correlate that with mitochondrial respiration from muscle biopsy samples assessed via seahorse (see methods below).

Results will provide significant understanding of mechanisms that underlie statin related mitochondrial dysfunction and impaired SkM performance and provide a potential avenue for an effective and safe treatment with Epi with the ultimate goal of increasing patient compliance and reducing CV mortality.

7. BACKGROUND AND SIGNIFICANCE

Statin therapy has revolutionized the treatment of CV disease. Since their approval from the Food and Drug Administration in 1987, statins have notably contributed to decreased mortality. Landmark clinical trials over the past decade, such as COURAGE, have shown that medical management of coronary artery disease (CAD), of which statins are the cornerstone of therapy, is as effective as percutaneous coronary intervention for

treatment of this disease.¹ The JUPITER trial demonstrated that even in healthy patients with normal lipid profile and baseline inflammation as assessed by elevated levels of high sensitivity C reactive protein (hsCRP) statins reduce mortality.² There are also emerging opinions stating that statins should be given to all patients over age 50 for primary prevention of CV disease.³

However, compliance with this life saving therapy can be problematic. The spectrum of statin related side effects ranges from severe myopathy (defined in clinical trials as muscle weakness with raised concentrations of creatinine kinase), which is rare to more common musculoskeletal side effects. These statin related muscle complaints include fatigue, pain, tenderness, weakness, nocturnal cramping, and tendon pain. Some patients on statins also complain of memory loss and decreased exercise tolerance. New controversial data has emerged suggesting that statins are associated with impaired glucose tolerance and new onset diabetes.^{4, 5} Up to 20% of patients prescribed statin therapy stop it due to these various side effects.⁶ It is even harder to have athletic patients take statins as they have significant muscular complaints and up to 80% of these patients cannot tolerate statin therapy.⁷ As the indications for statin therapy for primary prevention are growing, it is important to develop strategies that counteract the side effects of this therapy. There are alternatives to statins such as LDL apheresis and the emerging class of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, but these therapies have yet to demonstrate the mortality benefit that statins have and are very costly.

New data is emerging indicating that patients on statin therapy have decreased exercise performance and impaired mitochondrial function. Statins, which are hydroxyl-methylglutaryl coenzyme A reductase inhibitors, inhibit the production of mevalonic acid, which is a precursor in the synthesis of coenzyme Q10 (Coq10). Patients on statins have lower levels of Coq10 in serum and muscle, which is thought to be a mechanism of statin induced myopathies. However, in clinical trials, Coq10 supplements have not improved muscle function.⁸

In a recent study published in the Journal of the American College of Cardiology, Mikus et al, randomized 37 patients to 12 week of aerobic exercise training with or without statin therapy. In patients taking statins, exercise induced improvements in VO₂ max were not seen and SkM citrate synthase, which is a marker of mitochondrial content and function, decreased.⁹ Exercise stimulates mitochondrial biogenesis and mitochondrial function and these improvements were blunted in the patients taking statins.⁹ Other studies have shown that statin treatment results in impaired mitochondrial respiration mainly of complex I¹⁰ and increased reactive oxygen species (ROS) production and decreased oxidative capacity in SkM.¹¹ Interestingly, a study by Boutberi et al, indicates that statins have negative effects only in muscle and that statins improve oxidative capacity and decrease ROS production in the heart as assessed by tissue sampling of the human atrial appendage.¹¹ Hence, the importance of alleviating muscle related statin side effects and enhancing compliance with statins.

SkM biopsies from 11 patients with statin intolerance were compared with 7 controls. They showed impaired fatty acid oxidation (FAO) in patients that had statin intolerance.^{12,13} They also found increased RER (respiratory exchange ratio) in patients with statin associated muscle toxicity thought to be due to reduced fat oxidation (FAO). FAO occurs in mitochondria and these data implicate the mitochondria as therapeutic target to block statin associated toxicity.

Recent epidemiological studies have shown that the consumption of modest amounts of dark chocolate, which contains the natural cacao flavanol Epi, is associated with reductions in the incidence of CV diseases and improved blood pressure (BP) as well as cardiovascular and all-cause mortality.^{14,15} The health benefits of dark chocolate have been attributed to Epi.¹⁶ Clinical intervention studies using cocoa and/or dark chocolate in normal volunteers or subjects with cardiovascular diseases have reported improvements in peripheral and

coronary vascular endothelial function, BP, lipids, insulin resistance, and inflammatory markers.¹⁵ The mechanism underlying these improvements is thought to be due to increased nitric oxide levels and improved endothelial function.¹⁶

Our published studies with Epi (detailed below in preliminary results) were the first to suggest a new mechanism of action for Epi on mitochondria. We demonstrated that Epi has the capacity to improve mitochondrial structure and function in SkM and increase maximal mitochondrial respiratory rate.^{17,18} In a first-in-kind study in five patients with heart failure (HF) and type II diabetes (DM2), we demonstrated that in SkM biopsies there is a severe reduction in mitochondrial volume and cristae as well as structural/functional proteins in baseline skeletal muscle biopsies.¹⁷ After treatment with Epi enriched chocolate, there was a significant recovery of SkM mitochondrial cristae and structural/functional proteins. We also observed reductions in signaling molecules involved in mitochondrial biogenesis (MiB) and thus, muscle function including nitric oxide synthase (NOS), nitric oxide (NO), cyclic guanosine monophosphate (cGMP), silent mating type information regulation 2 homolog (SIRT1), peroxisome proliferator activated receptor- γ coactivator 1- α (PGC1 α), and transcription factor A mitochondrial (Tfam). These levels improved after treatment with Epi enriched chocolate.¹⁷

Based on these results we hypothesize that: *“Epi can block statin related detrimental effects on mitochondrial function and thus, preserve SkM structure/function. The mechanistic underpinnings of these functional improvements will likely be due to the upregulation of key MiB signaling pathways”.*

(-)-Epicatechin characteristics

(-)-Epicatechin is a naturally-occurring product found in chocolate and tea. It is a member of the flavonoid family. The (-)-epicatechin is purchased commercially and is obtained from tea extracts:

Manufacturer Sigma-Aldrich

Product # E1753

Country of Origin: India

It has >90% purity by HPLC, and has been purified in a GMP facility as follows: it is dissolved in ethanol, treated with charcoal, filtered to remove insolubles, the solvent is exchanged to purified water, and then the solvent is removed by lyophilization. The final test substance is tested in a GMP analytical lab using appropriate qualified HPLC methods for impurities, including chirality testing. Release specifications require > 90% purity, <5% of the enantiomer, and 5% of catechin. It is also tested for other characteristics typical in GMP materials (identity by ¹H NMR, IR; water content by Karl Fischer titration; ethanol content by GC; and the general USP tests of residue on ignition and heavy metals). Based on test results, a Certificate of Analysis is generated and a %-content by weight is calculated.

Chemical name: (-)-Epicatechin or (2R, 3R)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol

Molecular formula: C₁₅H₁₄O₆

CAS #: 490-46-0

Molecular weight: 290.27 grams/mole

Appearance: off-white to light brown solid

Solubility: >5 mg/ml in water at 20°C

pKa: neutral

Taste: moderately bitter, resembling aspirin in degree of bitterness

(-)-Epicatechin is a single enantiomer containing two defined chiral centers.

Stability (-)-Epicatechin has been demonstrated to be stable in the lyophilized state for at least three months

under refrigeration. A stability study is ongoing and retest dates will be generated based on results.

Administration: The intended route of administration is oral. For clinical trial usage, (-)-epicatechin is a supplied as preweighed drug substance in single-use, polypropylene bottles manufactured under United States current Good Manufacturing Practices. This powder-in-bottle supply is to be stored in a refrigerator or freezer until ready to use.

The UCSD compounding pharmacy will make epicatechin capsules for this study. Each capsule will contain 25 mg of epicatechin.

Sterility and pyrogen testing Sterility and pyrogen testing has not been conducted, as test product is supplied as lyophilized powder for oral use only. All products to be included in (-)-epicatechin formulation will be USP grade (vitamin C, EDTA, cellulose, lactose).

Safety of epicatechin

(a)Nonclinical Toxicology

Studies in rodents indicate that (-)-epicatechin can be delivered safely at doses far in excess of the anticipated 1mg/kg dose in human studies. In a study with green tea extracts containing (-)-epicatechin given orally to rats daily for 6 months, the no-observable-adverse-effect level (NOAEL) corresponded to 85 mg (-)-epicatechin/kg (38). In a developmental toxicity study in pregnant rats using a different tea extract, the no observable-adverse-effect level (NOAEL) corresponded to 100 mg (-)-epicatechin/kg (the highest dose tested).¹⁹ The intraperitoneal (-)-epicatechin median lethal dose (LD50) reported for mice is 1000 mg/kg as stated in the MSDS provided by suppliers such as Sigma-Aldrich. Clarke and Clarke proposed that any substance with an intraperitoneal LD50 of above 1000 mg/kg may be regarded as safe.²⁰

(b)Human Safety

Clinical studies with purified (-)-epicatechin (1-2 mg/kg) did not reported any adverse events. An extensive literature of human studies using high-flavanol cocoa or chocolate human studies exists, indicating that flavanols could be administered safely at doses up to 1008 mg flavanols per day for 15 days and 444 mg flavanols per day for 6 weeks.

A safety study of a green tea extract containing ~110 mg (-)-epicatechin per dose showed that once a day dosing for 4 weeks yielded the same safety profile as placebo, with no significant differences in hemotologic or clinical chemistry.²¹

Epidemiological studies have correlated flavanol-rich diets with improved cardiovascular prognosis and without any significant negative health issues.²² A recent meta analysis of interventional and observational studies encompassing ~114,000 subjects reports that the highest levels of chocolate consumption were associated with a 37% reduction in cardiovascular disease (relative risk 0.63 (95% confidence interval 0.44 to 0.90) and a 29% reduction in stroke compared with the lowest levels, with no increases in health risk factors.²³ A separate meta-analysis of studies examining the effects of chocolate on blood pressure (BP) found that dark chocolate significantly reduced blood pressure relative to controls, but only for hypertensive or prehypertensive subgroups.²⁴ BP was not significantly reduced in the normotensive subgroups, suggesting that flavanols including (-)-epicatechin do not override control of normal BP and do not pose a significant risk of producing hypotension. In addition to formal clinical studies, cocoa, chocolate and tea have been used globally on a daily basis for centuries, suggesting that these compounds pose no significant safety risk. Tea is considered a Generally Regarded As Safe (GRAS) compound by the FDA (21 CFR 182.20).

Recently collaborator Dr. Christopher Barnett at UCSF (UCSF IRB PROTOCOL 12-09455) completed

administration of pure epicatechin to healthy volunteers. Nine healthy participants were given a single dose of pure epi at 50, 100 or 200 mg with no adverse side effects. An additional 8 volunteers were given multi-dose epi at 50 mg daily (4 subjects) or 50 mg every 12 hours (4 subjects) for 5 days. There were no adverse effects noted. Based on this data and analysis of pharmacokinetics from this data the optimal dose appears to be 50 mg bid to achieve optimal pharmacokinetics (steady state levels of 200 nanomoles).

Metabolism and Pharmacokinetics of (-)-Epicatechin

(-)-Epicatechin is orally bioavailable, as the consumption of (-)-epicatechin or cocoa products can result in pharmacologically relevant levels of (-)-epicatechin and its metabolites in blood.

The absorption of flavonoids occurs mainly in the small intestine and takes place within minutes. In the small intestine, flavanols are extensively glucuronidated and partially methylated²⁵, allowing negligible amounts of native (-)-epicatechin in the mesenteric circulation. In the liver, further glucuronidation, methylation, and sulfation can take place.²⁶ Several studies have determined the presence of these conjugates in the plasma and urine of rodents and human²⁷, as well as in rat bile²⁸ and brain²⁹. In plasma, monomers are present as conjugated metabolites (90%) and nonconjugated molecules (10%)

Total plasma concentrations of (-)-epicatechin plus its metabolites are found in the low-micromolar range as soon as 1 h after the consumption of a flavanol-rich food (cocoa), with T_{max} ~2 hours.³⁰ The major metabolite of (-)-epicatechin detected in plasma is 4'-O-methyl-epicatechin-7-β-D-glucuronide.³⁰ The elimination half-time (t_{1/2}) of (-)-epicatechin and its metabolites from plasma ~2 hours, and over 90% of the urinary excretion of these compounds was complete by 8 hours.³¹

Oral bioavailability (peak plasma concentration/mg ingested) for (-)-epicatechin is ~15 nM/mg ingested. The consumption of a cocoa drink containing a total of 55 mg of (-)-epicatechin yielded at 2 h 0.63 M of total (-)-epicatechin, establishing a relationship of 11 nM/mg ingested.³² In a similar study a normalized level observed after a larger ingestion of (-)-epicatechin via a cocoa drink yielded a relationship of 15.7 nM/mg ingested.³³

For purified (-)-epicatechin (single oral dose, 200mg), exposure was 17.5 nM/mg ingested. For a 45 mg (-)-epicatechin dose in a green tea catechin mixture exposure was 19 nM/mg, representing ~1.1% injected dose.³⁴ The peak exposure of ~3.5 M total (-)-epicatechin compounds produced by the 200 mg oral dose is pharmacologically relevant, as administration of a lower dose (1 mg/kg body weight) resulted in significant improvement in vascular function (FMD).³⁵

8. PROGRESS REPORT

We had demonstrated in a 5-patient proof of concept clinical trial that epicatechin enriched chocolate (EEC) improves skeletal muscle indicators of mitochondrial biogenesis and structure in patients with heart failure (HF) type 2 diabetes (DM2).¹⁹ We also had demonstrated that epicatechin given by gavage (1 mg/kg BID) for 15 days to one year old male mice improved exercise performance as well as markers of mitochondria structure and biogenesis as well as capillarity in heart and skeletal muscle.¹⁸

9. RESEARCH DESIGN AND METHODS

Overall Study Design. The clinical trial will be registered under www.clinicaltrials.gov. Subjects between the ages of 30-75 who have LDL_≥100mg/dL, metabolic syndrome (must have Triglycerides_≥150 mg/dL), and VO₂ max >18 ml/kg/min will be enrolled. We are targeting patients with metabolic syndrome based on the results from our prior study in which we have shown that patients with metabolic syndrome had 26.5% reduction in triglycerides with 100 mg of epicatechin [40].

These are subjects who have not had any cardiovascular events and need statins for primary prevention. The study will be a double-blind placebo controlled study where subjects will be randomized to either of two

groups:

- 1) Simvastatin 40 mg + placebo twice daily (statin only group) for 3 months**
- 2) Simvastatin 40 mg + Epi 100 mg twice daily (statin + Epi group) for 3 months**

The 3 month time period will be used as prior literature and our results suggest that this length of therapy is needed to trigger changes in mitochondrial proteins that translate into functional endpoints.³⁶ The 50 mg daily dose for Epi was chosen based on our first preliminary results from the proof-of-concept study as we had shown that this dose is safe and associated with changes in biomarkers of mitochondrial functions and yield steady state levels of ~200 nM. However, when we conducted a preliminary analysis on the first 6 patients of this study, we realized that 50mg may not be a strong enough dose to notice changes in this specific patient population; thus, we are increasing the dose of Epi to 100mg daily. This is also supported by one of our other studies⁴⁰. We will obtain Epi powder (>98% purity, #E1753, Sigma-Aldrich) extracted from tea. Epi or placebo will be encapsulated by UCSD's investigational pharmacy using proper manufacturing practices. We have opted to use simvastatin at 40 mg/daily as this was the dose used by Mikus et al where impaired $\dot{V}O_2$ max was observed⁹ and is widely used in the cardiology community as the preferred generic statin. The study design will be adaptive and data analysis will be done in batches of 6 subjects (3 in each group) so that Epi dosing can be adjusted if needed. If the Epi dose needs to be adjusted, an amendment with a new dose will be submitted to the IRB. During the course of the treatments, subjects will be required to keep the same baseline level of activity and not to engage in any new exercise regimens. Participants will be provided with a list of foods to refrain from consuming, such as those high in Epi (e.g. green tea), during the duration of the study.

For patients who are currently on statins, there will be a sufficient washout period of 1-2 weeks prior to the start of the study. The washout period may come with the risks of cardiovascular events, heart attack and stroke. After the washout period, we may ask the patient to complete an exercise test to confirm that he/she meets the $\dot{V}O_2$ max >18 ml/kg/min inclusion criteria. The risks of exercise testing are explained below in Sections 14 and 15.

Participants will be contacted 1-2 times per month to assess their compliance with the capsule consumption, possible adverse effects and to review any dietary/lifestyle changes.

All patients will have blood tests to assess cardiometabolic health (lipids with NMR and LDL Direct, CMP, CBC, HBA1c, hsCRP, TSH, Apo A/B ratio) at the beginning and end of treatment. Epi levels will be checked in the statin + epi group after 2 weeks of treatment. This will be done by a non-blinded research assistant. The PI and others involved in data analysis will be blinded to who is the epi group and who is in the placebo group. If levels are not in the 200nM range, we will consider increasing or decreasing dosing and check patient compliance.

We will also assess platelet mitochondrial oxidative phosphorylation complex I, IV and citrate synthase activities and plasma follistatin, myostatin, and protein carbonyl levels. Additionally, we will assess nanoparticles.

Blood will be drawn before and after treatment. Muscle biopsies and exercise tests will be conducted in this manner as well. The muscle biopsies are an optional procedure and are not required for study participation.

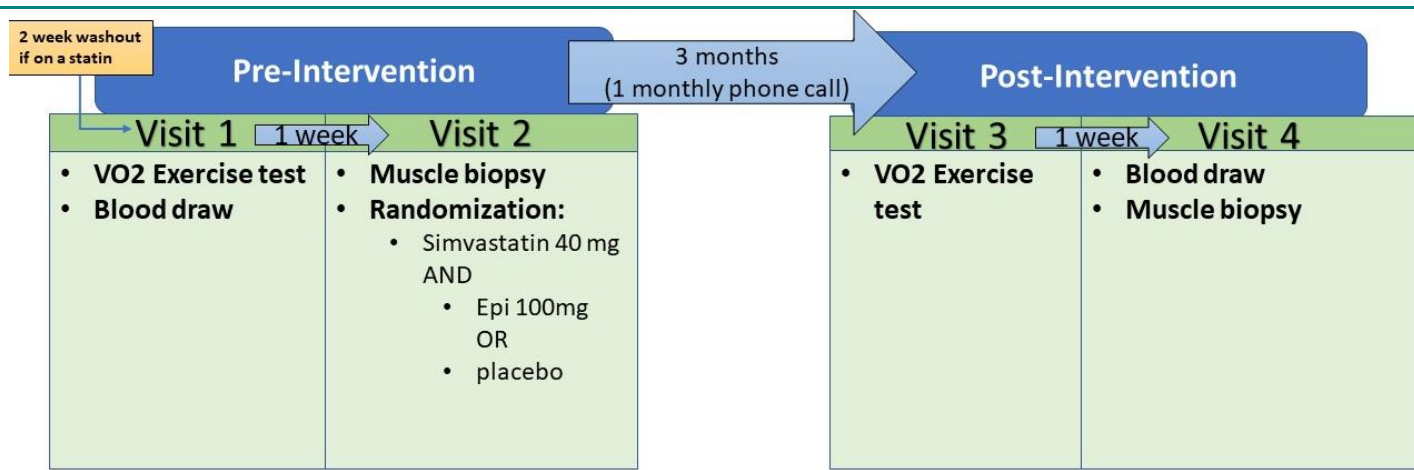


Figure 1. Study Design. Statin dose will be simvastatin 40 mg daily and epicatechin dose will be 100 mg. Muscle biopsy will be optional.

Statistical Analysis:

Power Analysis. Out of the planned 50 subjects, a drop-out rate of 10-20% is expected. Thus, we anticipate having 20 subjects per group (total of 40 subjects) available in the final analysis. The power analysis is based on a two-sample, two sided t-test comparing the difference in VO₂max between the three-month time point and baseline in the statin + placebo group vs the statin + Epi group. With 20 subjects in each group, the test has 80% power to detect an effect size as small as 0.909 ml/min/kg with a type-1 error rate of 0.05. The standard deviation of the change in VO₂ max was estimated based on prior studies to be 3.93.⁹ Therefore we can expect the test to detect a difference as small as $0.909 \times 3.93 = 3.57$ ml/min/kg in the change in VO₂ max between the two groups, with the given power and type-1 error.

Statistical Methods

The primary aim of the study is to test the effect of Epi treatment in statin users on VO₂ max. This will be tested at the 0.05 significance level using the t-test described in Aim 2 below. The other outcomes and tests in this study will be treated as secondary/exploratory; hence, unadjusted p-values will be reported for these tests. All tests will be two-sided.

Aim 1: The effects of the Epi treatment will be assessed by comparing the statin + placebo group and the statin + Epi group from the first stage of the study. For each outcome, we will test the null hypothesis of no difference between the two groups in the change in the outcome between baseline and the three month time point using a two-sample t-test.

Aim 2: In order to characterize the changes in mitochondrial function associated with statin use, we will analyze the data from the statin + placebo group. We will compare the variables of interest at baseline and the three month end point. For each variable, we will use a paired t-test to evaluate whether there is a significant change of measurement between baseline and end point.

Exploratory Aim 1 and 2: We will test for changes in blood-based biomarkers associated with statin + Epi treatment. Two-sample t-tests will be used as in Aim 1.

An initial evaluation using the first 6-10 subjects in the statin + placebo group will be made; based on these preliminary results, the inclusion criteria for the study may be adjusted. With these subjects, the paired sample t-test has 80% power to detect an effect size as small as 0.996 with a type-1 error of 0.05. Using the estimate of the standard deviation as in the power analysis above, we can expect the test to detect a change in VO₂ max

as small as $0.996 \times 3.93 = 3.914$ ml/min/kg in the statin + placebo group.

Specific Protocols. Brief synopsis of methods currently use in our laboratory (SkM biopsy, biochemical techniques including Westerns, ELISA, enzyme activities) is provided below and these are elaborated upon in our publications.¹⁷

Skeletal Muscle Biopsy: 1% lidocaine without epinephrine will be used for local anesthesia and percutaneous skeletal muscle biopsies will be obtained from the lateral portion of the quadriceps femoris using a 5mm diameter side-cutting needle to yield 200-300 mg of muscle. The muscle will be frozen in liquid nitrogen until analysis. Details on assessment of histology by EM is provided in prior publications.¹⁷

Western Blots: (SIRT1, PGC1 α , Tfam) Frozen skeletal muscle tissue (25 mg) will be homogenized and electrophoresed in poly acrylamide gels. Proteins will be transferred on to nylon membranes and incubated in sequence in primary and secondary antibodies. Enhanced chemiluminescence will be used for band detection and signals will be digitized using computer assisted densitometry. All signals will be normalized for differences in loading using GAPDH.

Citrate Synthase Assay: Tissues samples (25 mg) will be centrifuged and supernatants recovered. Citrate synthase activity will be measured using a kit assay (Sigma-Adlrch CS0720).

Epi Levels: 200 microliters of patient plasma will be precipitated using phosphoric acid in acetonitrile. Samples will be centrifuged and supernatant will be transferred to a separate tube and dried down. The sample will be dissolved in water with formic acid and injected in liquid chromatography column with metabolites detected HPLC mass spectrometry. Values will be calculated as per a spiked standard.

Blood Tests: The following blood tests will be processed at UCSD Clinical Laboratories. **1)** Cardiometabolic parameters: (lipids with NMR, LDL Direct, CBC, CMP, HBA1c, hsCRP, TSH, Apo A/B) **2)** Biomarkers Endpoints: follistatin, myostatin, protein carbonyl (ELISA Kit), ascorbyl radical, nanoparticles **3)** Platelet endpoints: platelet citrate synthase (see preliminary data), complex I/IV.³⁷

Cardiopulmonary exercise protocol (CPX): CPX with exhaled gas analysis will be performed after an overnight fast while subjects are on a constant diet and exercise regimen. Caffeine and alcohol will be prohibited for 18 hours before the test. The patient will be asked to exercise on a stationary bicycle. Breath by breath gas analysis will be collected during 30 minutes of rest and during ramped exercise on a stationary bicycle. The Orca[®] cardiopulmonary exercise testing system with an Oxigraf[®] laser diode absorption spectroscopy oxygen sensor and a CardioPulmonary Technologies[®] pulsed non-dispersive infrared spectroscopy CO₂ sensor will be calibrated with a known gas mixture before each gas collection. For each subject, we will measure indicators of aerobic function including VO₂ max, metabolic equivalent (METS), anaerobic threshold (AT) and the ventilatory efficiency (VE/VCO₂). We will assess recorded parameters of fuel selection such as the fasting RER.¹² The risks of the test include possible precipitation of cardiac arrhythmia or ischemia. The tests will be overseen by a cardiologist. If any medical issues are noted during the test, participants will be referred for prompt medical attention.

Methods for Mitochondrial respiration assay^{38,39}

Oxygen consumption rates (OCR) from a monolayer of adherent L6 cells will be measured using the Seahorse XF96 analyzer. The L6 cells will be seeded at 20,000 cells/well (passage 3-4) on a 96 well tissue culture plate (Seahorse Biosciences, North Billerica, MA, USA) 24h before the OCR measurements. The intact cells will then be incubated at 37°C for 1h with XF media supplemented with 10mM glucose, 1mM sodium pyruvate, 2mM L-glutamine, pH 7.4. The bioenergetic profile will be derived by acutely injecting control or patient

plasma (5%) through the port A followed by the modulators of the electron transport chain in form of drugs such as Oligomycin(1uM) to inhibit ATP synthase, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) (2uM) for maximal uncoupling and Rotenone/Antimycin A (0.5uM), a complex I inhibitor. The OCR will be calculated by plotting the O₂ tension of the media as a function of time (pmol/min). Also, 50uL of plasma will be used in Electron paramagnetic resonance (EPR, Magnettech, Berlin, Germany) to measure Ascorbyl Radical.

Aim 1: Assess the added effects of Epi in reducing triglyceride levels, and in improving/maintaining VO₂ max in patients with metabolic syndrome.

Rationale: Based our prior research and the preliminary results from the first 6 patients that patients in the Epi +statin group will have significantly lower triglyceride levels compared to those in statin only group.

Protocol: All subjects will have blood drawn (including lipid panel) before and after the intervention period.

Endpoints to assess:

- Triglyceride levels

Expected Results: We expect in the statin + Epi group that Triglyceride levels will decrease (improve).

Aim 2: Characterize statin associated changes in functional status in patients with metabolic syndrome starting statin therapy.

Rationale: We believe that patients with metabolic syndrome have more metabolic stress and mitochondrial dysfunction allowing to see greater differences in our end points.

Protocol: Twenty subjects will be randomized to the statin only group and take statins + placebo for 3 months, and thirty subjects will be randomized to the statin + Epi group for 3 months. Subjects will undergo fasting CPX protocol followed by breath gas analysis during a ramped exercise bicycle protocol, blood draw, and optional muscle biopsy at beginning and at the end of treatment.

Endpoints to assess

CPX testing will test for:

- Functional Capacity as assessed by $\dot{V}O_2$ max, (primary outcome).
- Ventilatory efficiency (VE/ $\dot{V}CO_2$).
- Fasting Respiratory exchange ratio (RER).
- Metabolic equivalent (METS)

Functional mitochondrial testing will include:

- Oxygen consumption rates as assessed by Seahorse analyzer from skeletal muscle biopsy

Expected results: We anticipate that subjects will exhibit a moderate decrease in SkM strength, $\dot{V}O_2$ max, VE/ $\dot{V}CO_2$, and increased METS and RER following statin therapy as our subject recruitment is biased towards active individuals who tend to be sensitive to statin associated decreases in exercise capacity.⁷ We also expect to see changes in mitochondrial function to reflect abnormal MiB.

Exploratory Aim 1: Assess the added effects of Epi in reducing LDL levels.

Rationale: Based our prior research and the preliminary results from the first 6 patients that patients in the Epi +statin group will have significantly lower LDL levels compared to those in statin only group.

Protocol: All subjects will have blood drawn (including lipid panel) before and after the intervention period.

Endpoints to assess:

- LDL levels

Expected Results: We expect in the statin + Epi group that Triglyceride levels will decrease (improve).

Exploratory Aim 2: Interrogate for possible blood-based biomarkers that correlate with changes in muscle biopsy.

Rationale: Results from prior studies indicate that there are changes in the blood that correlate with changes in mitochondrial respiration.

Protocol: We will be performing the abscorbyl radical assay from plasma (which is indicator of oxidative stress) and correlate that with mitochondrial respiration from muscle biopsy samples assessed via seahorse (see methods below).

Changes in these endpoints will be correlated with those obtained from biopsy results with the goal of developing non-invasive assays to assess Epi effects on SkM.

The remaining samples after analysis, including the skeletal muscle samples, will be stored indefinitely at UCSD for unforeseen future analysis as funding becomes available. Potential future research studies could include exploring additional biomarkers that possibly correlate with +/- Epi induced changes in SkM mitochondrial structure/function. Dr. Taub will have solitary control of the stored specimens and samples will only be shared with authorized research personnel under Dr. Taub's discretion. The Moore clause will be implemented regarding possible DNA analysis as reflected in the consent. To minimize the potential loss of confidentiality, patients will be assigned a unique number as their subject identifier code. The unique subject code will be used to label the samples. The key that relates the code numbers to the individuals will be kept in a locked cabinet in Dr. Taub's office.

All procedures mentioned above are research procedures and are not part of standard care.

Results will not be used for diagnosis, treatment, and prevention of disease in patients.

Incidental finding that have resulted from the conduct of this study or those that are found through lab testing for this study that require medical intervention for the subject will be noted by the PI or her project's medical research staff. They will refer the subject to his primary care physician. If the subject does not have a primary care physician, a suggested list of doctors will be given to him or her to choose from.

10. HUMAN SUBJECTS

Recruitment: We will recruit 50 adult (30-75 years) volunteers from the cardiology and internal medicine clinics at UCSD Medical Center to complete the study. Also, we will be utilizing the bioinformatics report from EPIC to help recruit patients who may qualify for our inclusion criteria. We will be recruiting patients with the following conditions: metabolic syndrome (must have triglycerides > 150mg/dL), LDL >100 md/dL, and VO₂ max >18 ml/kg/min. Each of these patients will be randomized into the two groups mentioned above. Review of the cardiology clinic patients over the past 5 months reveals that there were 50 subjects that would meet our inclusion/exclusion criteria. We anticipate a 10-20% subject drop out based on our experience with prior studies. With the drop out, we anticipate having 20 subjects per randomized group. If any patients decide to withdraw from the study due to the intolerance of statins, we will try to obtain a muscle biopsy (if they underwent a baseline muscle biopsy), blood test and exercise test at the time of drop out for endpoint

measurements.

All patients will have blood tests to assess cardiometabolic health (lipids with NMR, LDL direct, CMP, CBC, HBA1c, hsCRP, TSH, Apo A/B ratio) at the beginning and end of treatment. Epi levels will be checked in the statin + epi group after 2 weeks of treatment. If levels are not in the 200nM range we will consider increasing or decreasing dosing and check patient compliance. Blood will be drawn before and after treatment.

Subjects will be recruited from UCSD clinics and ResearchMatch. The main sites of the clinical testing will be conducted at:

1) UCSD's Altman Clinical and Translational Research Institute (ACTRI) for vitals, muscle biopsy, blood draw, exercise test.

Inclusion/exclusion criteria for studies in normal subjects are:

1) Inclusion:

- a. Subjects aged 30-75.
- b. Subjects must have VO2 max greater than 18 ml/kg/min
- c. Subjects with LDL > 100 mg/dL
- d. Subjects with Metabolic Syndrome is defined as:
 - a. Triglycerides ≥ 150 mg/dL and < 500 mg/dL (or on drug treatment for elevated triglycerides) and 2 out of 4 of the following:
 - i. Waist circumference ≥ 102 cm (Men) or ≥ 88 cm (Women).
 - ii. Elevated fasting glucose ≥ 100 mg/dL (or drug treatment of elevated blood glucose).
 - iii. Reduced HDL-C < 40 mg/dL (Men), < 50 mg/dL (Women) (or on drug treatment for reduced HDL-C).
 - iv. Elevated blood pressure, systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg (or treatment with antihypertensive drug with history of hypertension).
- e. Subjects without a prior cerebrovascular event (example: stroke)
- f. Subjects without a prior cardiovascular event (example: heart attack)

2) Exclusion:

- a. Subjects with liver disease (due to genetics, alcohol, infections, or cirrhosis) or kidney disease (due to decreased or impaired kidney function).
- b. Subjects taking drugs that interfere with statin metabolism (calcium channel blockers, colchicine etc.)
- c. Acute or chronic infectious disease; autoimmune/inflammatory disease, cancer, COPD, anemia, insulin-dependent diabetes, or psychiatric illness.
- d. Non-smokers, prior smokers, or illicit drug users with abstinence > 1 year.
- e. For participants electing to do the muscle biopsy procedure only: Subjects taking blood thinners (Coumadin, xarelto, pradaxa, Plavix, effient, epixiban etc) will be excluded. If subjects are taking aspirin for routine prevention they will be asked to hold aspirin for 7 days prior to biopsy. Routine prevention means that the patients are taking aspirin to reduce their risks, but do not have established cardiovascular disease.
- f. History of previous knee surgery and active history of knee pain or neuromuscular disease are exclusions as they are known to affect muscle structure and function.

11. RECRUITMENT

We will be screening and recruiting patients through ResearchMatch, which enables researchers to search for appropriate matches in non-identifiable volunteer profiles. We will also screen and recruit normal subjects and patients from UCSD. We will be screening patients through EPIC, with the help of their bioinformatics department, for research subjects who might qualify for our criteria. EPIC Bioinformatics will help search for the following to generate a list of potential research subjects:

1. Patient's name, date of birth (DOB), and Medical Record Number (MRN)
2. Patient's primary care provider (PCP) name and contact information
3. LDL>100mg/dL for inclusion
4. Triglycerides $\geq$ 150 mg/dL for inclusion
5. Metabolic syndrome for inclusion
6. Ages 30-75 for inclusion.
7. No prior adverse cardiovascular events (ex: heart attack) for inclusion.
8. No prior cerebrovascular events (ex: stroke) for inclusion.
9. History of liver or kidney disease for study exclusion.
10. Current use of drugs that interfere with statin metabolism (calcium channel blockers, colchicine, etc.) for study exclusion.
11. History or chronic infectious disease; autoimmune/inflammatory disease, cancer, COPD, anemia, insulin-dependent diabetes, or psychiatric illness for study exclusion.
12. History of smoking and/or illicit drug use for study exclusion.
13. Current use of blood thinners (Coumadin, xarelto, pradaxa, Plavix, effient, epixiban) for study exclusion.
14. History of previous knee surgery and active history of knee pain or neuromuscular disease for study exclusion.

After we identify patients of interest for the study, we will contact their primary care provider and get their approval to contact the patient. We will inform clinicians in the department of endocrinology, cardiology family medicine and internal medicine of our study. We will ask these clinicians to ask their patients if they are interested in a study regarding their HF and exercise capacity. We will also ask the clinicians to specifically ask the interested patients if it is acceptable if one of our coordinators contact them. If the patients are interested and give their consent then our study coordinator will either contact them by phone or after their clinic visit.

At UCSD, subjects will be recruited from UCSD Sulpizio Cardiovascular Center Clinics, UCSD Internal Medicine Clinic in La Jolla and UCSD Endocrinology Clinic in La Jolla. We will be screening patients prior to informed consent. The following are justifications for partially waiving the HIPAA for screening purposes:

1. It allows the timely review of PHI for patients who are visiting the UCSD clinics, which will allow a greater chance of finding eligible patients. It is imperative that we consent the right patients for the study in order to:
 - a. Reduce the probability of screen failures, and minimize the waste of resources, time and funds.
 - b. Conduct research more smoothly and efficiently, without having to unnecessarily disturb patients who do not qualify for our study.
2. We will be using the partial HIPAA waiver only for the purposes of determining the eligibility of patients, and nothing more. This information can only be determined by screening the patients' medical records on Epic.
3. Screening cannot be practicably conducted without the use of PHI because subject eligibility depends on

inclusion and exclusions factors that can only be found in their medical records. The PHI we will be using during the screening process are the patient's age, sex, past medical history, active problems, procedures, images, and medications. Only the PI and the research team will have access to this information.

4. There will be no disclosure of data to anyone outside this research group. PHI will be protected from improper use/disclosure. This will be done by securing the PHI in a locked cabinet in the PI's locked office at the Clinical Translational Research Institute (CTRI). Only the PI and the research team will have the keys to the office and the cabinet so that only they can review the documents. The PHI will never leave the office or the building.
5. The use of PHI by our research team does not involve more than minimal risk since there are no routine physical or psychological examinations or tests for screening.
6. The research could not be practicably conducted without the waiver since we have to screen the subject prior to recruiting them into the study.
7. The privacy risks are reasonable relative to the anticipated benefits of the research, since the results of the study may improve patient compliance in taking statins, an important drug for cardiovascular disease.
8. If the patient does not qualify, or does not agree to participate in the study, his/her PHI will not be re-used and will be destroyed at the earliest opportunity.

12. INFORMED CONSENT

See attached consent.

Informed consent will be obtained by the study coordinators or research assistants associated with this protocol. Informed consent procedures will be supervised either by the study coordinator, supervising physicians, or the principal investigator. All research personnel giving informed consent will have undergone proper training and obtained the required certificates.

The following are justifications for **partially waiving informed consent** for screening purposes:

1. It allows the timely review of PHI for patients who are visiting the UCSD clinics, which will allow a greater chance of finding eligible patients. It is imperative that we consent the right patients for the study in order to:
 - a. Reduce the probability of screen failures, and minimize the waste of resources, time and funds.
 - b. Conduct research more smoothly and efficiently, without having to unnecessarily disturb patients who do not qualify for our study.
2. **Minimal Risk:** The screening procedures are considered minimal risk to the potential subjects because we will be using the partial consent waiver only for the purposes of determining the eligibility of patients, and nothing more. This information can only be determined by screening the patients' medical records on Epic. Screening **cannot be practicably conducted without the use of PHI** because subject eligibility depends on inclusion and exclusions factors that can only be found in their medical records. The PHI we will be using during the screening process are the patient's age, sex, past medical history, active problems, procedures, images, and medications. Only the PI and the research team will have access to this information. There will be no disclosure of data to anyone outside this research group. PHI will be

protected from improper use/disclosure. This will be done by securing the PHI in a locked cabinet in the PI's locked office at the Clinical Translational Research Institute (CTRI). Only the PI and the research team will have the keys to the office and the cabinet so that only they can review the documents. The PHI will never leave the office or the building. The use of PHI by our research team also does not involve more than minimal risk since there are no routine physical or psychological examinations or tests for screening. If the patient does not qualify, or does not agree to participate in the study, his/her PHI will not be re-used and will be destroyed at the earliest opportunity.

3. **Rights and Welfare of Subjects:** The waiver of consent would not adversely affect the rights and welfare of the potential subjects. Their standard of care in the hospital will remain the same regardless of the partial waiver of consent.
4. The potential subject will be informed about the purpose of the study, the duration of the study, the activities they will be doing in the study, the risks and benefits, expenses, compensation, alternatives to participating, privacy, their rights as research subjects and study contact information.
5. The privacy risks are reasonable relative to the anticipated benefits of the research, since the results of the study may improve patient compliance in taking statins, an important drug for cardiovascular disease.

Reconsenting

In the event that the consent is amended, participants who are active and have not yet completed the study will be informed of these changes and be asked to sign the updated consent form. The updated consent form will be provided to them at an upcoming visit, or through mail, email or fax. They will be given time to review these changes and provide their written consent. They may sign and return it to the research team at the upcoming visit, or through mail, email or fax.

Participants who have completed the study and are no longer active in the study will be made aware of the changes through mail, email or fax. However, these participants will not be asked to acknowledge or confirm the receipt of the updated consent or to sign and return it.

13. ALTERNATIVES TO STUDY PARTICIPATION

Subjects have the right to refuse participation in the study. Epicatechin is a natural component of dark chocolate and is not part of patient's standard medical regimen. Patients will have no changes to their current medical regimen. They can choose to stop taking epi at any time.

14. POTENTIAL RISKS

1. Venipuncture: pain, a bruise at the point where the blood is taken, discoloration, redness and swelling of the vein and infection.
2. Cardiopulmonary exercise test: arrhythmia, low blood pressure, chest pain, fainting, heart attack.
3. Muscle Biopsy: include a certain degree of pain during the procedure, hematoma formation and a small residual scar. Some discoloration or bruising is also possible after the adipose tissue biopsy. The risks of hematoma formation are minimized by pressure and strict bed rest for 1-2 hours following these procedures. The Principle-Investigator, Dr. Taub has performed over 40 muscle biopsies in the pilot study and under UCSD IRB 111680 without any complications. Subjects will be thoroughly informed as to these potential problems. There is a very small chance that the scars from the biopsy sites may hypertrophy. There are also small risks of swelling and infection.
4. Consuming epicatechin: allergy
5. Consuming Statins: myositis, myalgia, recurrent muscle pain, rhabdomyolysis
6. Statin Washout Period: This 1-2 week washout period may increase risks of cardiovascular events, such as

heart attack or stroke.

7. A potential loss of confidentiality: The UCSD Institutional Review Board (IRB), the FDA, and other government agencies may inspect the study records. To minimize the risk of a potential loss of confidentiality, subjects will be assigned a unique subject code and their blood samples will be assigned a different unique specimen code. A separate paper document will link patient identifiers with subject codes and specimen codes. This document will be kept in a secure location in a locked cabinet. Only the PI and study coordinator will have the key to the cabinet. Some research documents may have certain patient identifiers (such as their name, medical record number, date of birth, race, ethnicity, age, and gender) in addition to their unique study ID, for the purposes of correctly identifying patients in the data analysis process and for correctly interpreting the data as parameters such as gender and age can influence results. However, this will be kept to a minimum and confidentiality will be held in the highest of regards.

15. RISK MANAGEMENT PROCEDURES AND ADEQUACY OF RESOURCES

Strict sterile technique will be observed during venipuncture and muscle biopsy.

The risks of taking blood include pain, a bruise at the point where the blood is taken, discoloration, redness and swelling of the vein and infection. Pressure will be held at the site of venipuncture/biopsy to minimize bruising, blood loss and swelling. If patients are anemic with hemoglobin 10 they will be excluded from the study.

All subjects are screened with a medical history and physical exam to ensure none has health problems that put them at risk. To minimize bleeding complications, coagulation parameters must be normal for participation. Blood drawing will be minimized to no greater than 200 ml per patient. Subjects will be thoroughly informed as to these potential problems.

Because this is a low risk study, a Data Safety Monitoring Board (DSMB) is not necessary. Instead, a Data Safety Monitoring Plan (DSMP) is provided.

A DSMB is not needed for this study for various reasons:

1. The study meets the following IND exemption criteria: if the clinical investigation is intended only to evaluate the dietary's supplement effect on the structure or function of the body and *not* to evaluate the supplement's ability to diagnose, cure, mitigate, treat or prevent a disease (**see attached Supplement to Biomedical Application Research Plan IND Exemption**). According to the FDA guidelines, the epicatechin is a dietary supplement: "...a *dietary supplement* is defined, in part, as a product taken by mouth that is intended to supplement the diet and that contains one or more dietary ingredients. The dietary ingredients in these products can include vitamins, minerals, herbs and other botanicals, amino acids, other dietary substances intended to supplement the diet, and concentrates, metabolites, constituents, extracts, or combinations of the preceding types of ingredients. Dietary supplements can be found in many forms such as tablets, capsules, softgels, liquids, or powders...a dietary supplement is not considered a drug and is not subject to the premarket approval requirements for drugs if the intended use for which it is marketed is only to affect the structure or any function of the body (i.e., not intended to be used for a therapeutic purpose).
2. This is a low risk study assessing the efficacy of a natural product classified by the FDA as a food substance given in dosages encountered in commercially available food products.
3. This study is intended to evaluate the dietary supplement's effect on the structure or function of the body and not to evaluate the supplement's ability to diagnose, cure, mitigate, treat or prevent disease.
4. The study does not involve a high-risk intervention or a vulnerable population, so DSMB should not be needed.
5. This is a single site study that is **not intended** to evaluate treatments intended to prolong life or reduce

-
- risk of a major adverse health outcome.
6. This study is **not intended** to compare rates of mortality or major morbidity.
 7. This study is addressing lesser outcomes, such as improving patient compliance.
 8. The study population is not at an elevated risk of more severe outcomes.

DATA SAFETY MONITORING PLAN (DSMP)

Oversight responsibilities

Oversight of the trial is provided by the Principal Investigator (PI), Dr. Taub.

Monitoring procedures

Dr. Taub assures that informed consent is obtained prior to performing any research procedures, that all subjects meet eligibility criteria, and that the study is conducted according to the IRB-approved research plan. Study data are accessible at all times for the PI to review. The PI reviews study conduct such as accrual, drop-outs, protocol deviations on a monthly basis. The PI reviews AEs individually real-time and in aggregate on a weekly basis. The PI reviews serious adverse events (SAEs) in real-time. The PI ensures all protocol deviations, AEs, and SAEs are reported to the IRB according to the applicable regulatory requirements.

Collection and reporting of SAEs and AEs

For this study, the following standard AE definitions are used:

Adverse event: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure.

Serious Adverse Event: Any AE that results in any of the following outcomes:

9. Death
10. Life-threatening
11. Event requiring inpatient hospitalization or prolongation of existing hospitalization
12. Persistent or significant disability/incapacity

AEs are graded according to the following scale:

Mild: An experience that is transient, & requires no special treatment or intervention. The experience does not generally interfere with usual daily activities. This includes transient laboratory test alterations.

Moderate: An experience that is alleviated with simple therapeutic treatments. The experience impacts usual daily activities. Includes laboratory test alterations indicating injury, but without long-term risk.

Severe: An experience that requires therapeutic intervention. The experience interrupts usual daily activities. If hospitalization (or prolongation of hospitalization) is required for treatment it becomes an SAE.

The study uses the following AE attribution scale:

Not related: The AE is clearly not related to the study procedures (i.e., another cause of the event is most plausible and/or a clinically plausible temporal sequence is inconsistent with the onset of the event).

Possibly related: An event that follows a reasonable temporal sequence from the initiation of study procedures, but that could readily have been produced by a number of other factors.

Related: The AE is clearly related to the study procedures.

AEs are identified immediately after the study procedure. We will have the patient rest for 30 minutes to an

hour after the procedure or activity to observe him or her for any AEs. We will follow up with patient the next day and once a week for the following weeks leading up to their next appointment with us. Dr. Taub who is a cardiologist and a team of nurses will always be nearby the patient at the appointment in case an event occurs.

SAEs and specific procedure-associated AEs are reported to the PI and IRB within 24 hours. In addition, all AEs are reported according to the IRB AE reporting guidelines.

Management of risks to subjects

Expected AEs

- 13. Blood draw
 - a. Hematoma
 - b. Arterial puncture
 - c. Pain
 - d. Nerve damage
 - e. Re-bleeding
 - f. Allergy
 - g. Phlebitis
 - h. Vasovagal reaction
 - i. Anxiety/fear
- 14. Muscle biopsy
 - a. Bleeding
 - b. Hematoma
 - c. Pain
 - d. Edema
 - e. Inflammation
 - f. Erythema
 - g. Ecchymosis
 - h. Panic
 - i. Numbness
 - j. Denervation
 - k. Atrophy
 - l. Abnormal scarring
 - m. Infection
- 15. Cardiopulmonary exercise test
 - a. Arrhythmia
 - b. Low blood pressure
 - c. Chest pain
 - d. Fainting
 - e. Heart attack
- 16. Consuming Epicatechin
 - a. Allergy
- 17. Consuming Statins
 - a. Myositis
 - b. Myalgia
 - c. Recurrent muscle pain
 - d. Rhabdomyolysis

18. Blood Draw:

- a. Prior to enrollment into the study, the patient will be educated on the risks of the blood draw. The phlebotomist will calm the patient beforehand to reduce anxiety/fear and minimize the probability of vasovagal response. Only sterile needles and gauze will be used to prevent allergic reactions and infection. The area of the blood draw will be thoroughly sanitized with an alcohol wipe prior to the needle stick. Care will be used when penetrating the skin with the needle so that there is a minimized risk of punctured artery and damaged nerve. The needle stick will be done swiftly and securely by an experienced phlebotomist in order to reduce pain. Pressure will be applied on the area immediately after the needle stick to minimize the risk of hematoma. The phlebotomist will inform the patient on when and how to undress their puncture site. Dr. Taub, who is a cardiologist, will be overseeing the procedure to check for the patient's vital signs. Dr. Taub's phone number will be given to the patient for any questions or concerns that may arise in the future.
- b. In the event of an AE that occurs during the appointment, the facility (CTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Taub and the nurses will be nearby to administer immediate medical care and send the patient to the nearest hospital. If the AE occurs after the appointment, the patient will be referred for prompt medical attention. The patient will be withdrawn from the study. Dr. Taub will monitor the patient until the problem has been resolved or has stabilized.

19. Muscle biopsy

- a. Prior to enrollment into the study, the patient will be educated on the risks of getting a biopsy from their leg muscle. Dr. Taub or Dr. Wilkinson will calm the patient beforehand to reduce anxiety/fear and minimize the probability of vasovagal response. Only sterile needles and gauze will be used to prevent allergic reactions and infection. The area of the muscle biopsy will be thoroughly sanitized with an alcohol wipe prior to the incision with scalpel and the needle stick. Before the procedure is performed an area of skin and the deeper tissues on the outer front portion of either one of the patient's lower thighs will be numbed using anesthetic (xylocaine) and then the small incision will be made with the scalpel. The special biopsy needle will be inserted through the incision and into the thigh muscle with extreme care several times and a small amount of tissue will be removed (about the size of a small pea). Deep pressure will be applied on the area for 20 minutes to minimize the risk of bleeding and then a steri-strip tape will be used to close the incision. Dr. Taub or Dr. Wilkinson, who are cardiologists, will perform the procedure. Dr. Taub has performed over 200 muscle biopsies with no complication. Dr. Taub's phone number will be given to the patient for any questions or concerns that may arise in the future.
- b. In the event of an AE that occurs during the appointment, the facility (CTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Taub and the nurses will be nearby to administer immediate medical care and send the patient to the nearest hospital. If the AE occurs after the appointment, the patient will be referred for prompt medical attention. The patient will be withdrawn from the study. Dr. Taub will monitor the patient until the problem has been resolved or has stabilized. The patient's conditions will be reported back to the PI.

20. Cardiopulmonary Exercise Test

- a. Prior to enrollment into the study, the patient is educated on the risks of the test. On the day of the test, the patients are asked to bring in their current medications in case any events that occur require them to take their medications. Before the test, the patient is told to signal to the research team and physicians with his hands if he begins feeling uncomfortable during the test.

The test will be stopped immediately to prevent exacerbation of their condition. The test will be overseen by Dr. Taub, a cardiologist. A research person will follow up with the patient the next day and once a week for the next 90 days to assess the patient's condition. The patient's conditions will be reported back to the PI.

- b. In the event of an AE that occurs during the appointment, the facility (CTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Taub and the nurses will be nearby to administer immediate medical care and send the patient to the nearest hospital. If the AE occurs after the appointment, the patient will be referred for prompt medical attention. The patient will be withdrawn from the study. Dr. Taub will monitor the patient until the problem has been resolved or has stabilized.

21. Consuming Epicatechin

- a. Dr. Taub, a cardiologist, will give the patients specific instructions on the amount of epicatechin capsules they would need to consume each day. A research person will follow up with the patient the next day and once a week for the next 90 days to assess the patient's condition. The patient's conditions will be reported back to the PI. If the patients have reasons to believe that epicatechin is causing any discomfort, consumption of the capsule will be stopped.
- b. In the event of an AE that occurs during the appointment, the facility (CTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Taub and the nurses will be nearby to administer immediate medical care and send the patient to the nearest hospital. If the AE occurs after the appointment, the patient will be referred for prompt medical attention. The patient will be withdrawn from the study. Dr. Taub will monitor the patient until the problem has been resolved or has stabilized.

22. Consuming Statins

- a. Dr. Taub, a cardiologist, will give the patients specific instructions on the amount of statins they would need to consume each day. If the patient patients have reasons to believe that statins are causing any discomfort, they will be stopped from taking the statins any further so as to prevent any events from occurring. A research person will follow up with the patient the next day and once a week for the next 90 days to assess the patient's condition. The patient's conditions will be reported back to the PI.
- b. In the event of an AE that occurs during the appointment, the facility (CTRI) is equipped with trained medical staff (physicians and nurses) who can respond quickly. Should an event occur, Dr. Taub and the nurses will be nearby to administer immediate medical care and send the patient to the nearest hospital. If the AE occurs after the appointment, the patient will be referred for prompt medical attention. The patient will be withdrawn from the study. Dr. Taub will monitor the patient until the problem has been resolved or has stabilized.

23. Statin Washout Period

- a. Dr. Taub will monitor the patients closely during this washout period. Dr. Taub will remove patients from the study if it is in their best medical interest. A research person will follow up with the patient once a week during this period to assess the patient's condition. The patient's conditions will be reported back to the PI.
- b. If an AE occurs, the patient will be referred for prompt medical attention. The patient will be withdrawn from the study. Dr. Taub will monitor the patient until the problem has been resolved or has stabilized.

In the event that a patient either withdraws from the study or the investigator decides to discontinue a patient due to SAE, the patient will have appropriate follow-up medical monitoring. Monitoring will continue until the problem requiring hospitalization has resolved or stabilized with no further change expected, is clearly unrelated to study medication, or results in death.

Plan for data management

Data will be collected using standardized paper forms and will only be identified with the study's ID of the participant. The codes that link the name of the participant and the study ID will be kept confidential by the Principal Investigator in a secured cabinet. Data will be entered in the computer independently by UCSD certificated and trained data entry staff, and discrepancies corrected by a supervisor based on source documents.

An internal data quality process will involve the PI performing a random 10% data audit twice per year. The PI will document and discuss findings with the research team and provide additional training, if necessary.

16. PRIVACY AND CONFIDENTIALITY CONSIDERATIONS INCLUDING DATA ACCESS AND MANAGEMENT

All study forms including consents, HIPAAs, case report forms (CRFs), and source documents which will include elements from the present and past history, patient demographics, including age, current medications and lab draws, will be stored in a locked cabinet in Dr. Taub's office. Only approved study personnel will have access to this information. To minimize the potential loss of confidentiality, patients will be assigned a unique number as their subject identifier code. The unique subject code will be used to label all study documents, in addition to certain patient identifiers as it relates to data analysis and data tracking. This is in accordance with privacy guidelines as these files are kept secure, private, and confidential in a locked cabinet in Dr. Taub's locked office.

Some portions of the specimens collected may be sent to a central laboratory outside of UCSD for testing. All samples will be labeled with a unique specimen code and the only way to link the specimens with the subject code and any personal health identifiers will be on a physical sheet of paper locked in a cabinet in Dr. Taub's office.

Social security numbers will be collected only for purposes of subject payment. This information will only be available to study personnel and will be secured in the same manner mentioned above.

17. POTENTIAL BENEFITS

Subjects may or may not benefit directly from participating in the study. The potential benefits to society in general include improved understanding of mitochondrial function in heart failure. The increased knowledge may lead to the design of more effective therapeutic regimens for heart failure.

18. RISK/BENEFIT RATIO

Statins are an iconic drug class that has improved mortality in patients with cardiovascular disease. However, up to 20% of patients who must be on this important drug do not take it due to skeletal muscle (SkM) associated side effects that range from muscle cramps to fatigue. If epicatechin can help in these conditions it can open up many different possibilities for interventions and treatments. We believe that risks are minimal in this study.

19. EXPENSE TO PARTICIPANT

There will be no expense to participants. The epicatechin for the study will be provided to all patients at no charge.

20. COMPENSATION FOR PARTICIPATION

Patients will be compensated \$300 for fully participating in the study. \$150 will be dispensed when beginning the study, and the remaining \$150 will be given upon completing the study. In addition to the \$300 compensation, the patients will be eligible to receive \$20 for each additional blood draw visit (\$80 maximum for 4 visits).

Patients who opt not to participate in the muscle biopsy will receive \$100 total. \$50 will be dispensed when beginning the study and the remaining \$50 will be given upon completing the study.

21. PRIVILEGES/CERTIFICATIONS/LICENSES AND RESEARCH TEAM RESPONSIBILITIES

Dr. Pam Taub is an Associate Professor of Medicine at UCSD and is board certified in internal medicine and cardiology. She has clinical privileges at the VA Medical Center and UCSD Medical Center. She will be responsible for all aspects of the clinical trial including project design, oversight of project and study personnel, patient recruitment, IRB submissions, data analysis, and manuscript writing.

Dr. Francisco Villarreal is a Professor of Medicine and will be responsible for analysis of epicatechin levels and analysis of muscle biopsy specimens.

Dr. Hemal Patel is a Professor in the Department of Anesthesiology at UCSD. He will be responsible for biomarker analysis (i.e. ascorbyl radical).

Michael Wilkinson, MD is an Assistant Professor of Medicine at UCSD and is a board-certified cardiologist. He will assist with the following: project design, patient recruitment, data analysis and manuscript writing.

Jia Shen, MD is an Assistant Professor of Medicine at UCSD and is a board certified cardiologist. She will assist with the following: project design, patient recruitment, data analysis and manuscript writing.

Cindy Knott is a registered dietician at CTRI and will be assisting with exercise testing.

Trained ACTRI nurses will assist with the muscle biopsy procedure.

Adena Zadourian and Hannah Lo are research coordinators who will who will be involved with overseeing the research assistants, administrative support, consenting subjects, as well as participating in data collection and analysis. They have undergone proper training and obtained the certificates necessary to work with human subjects.

Ashley Rosander, Aryana Pazargadi, Nikita Mittal, and Cameron Ormiston are research assistants who will be involved with administrative support, consenting subjects, as well as participating in data collection. They have undergone proper training and obtained the certificates necessary to work with human subjects.

Dr. Steven Moyo is a fully licensed and practicing internal medicine hospitalist at UCSD and will be involved with the recruitment process.

All blood draws will be performed by research personnel trained in phlebotomy.

22. BIBLIOGRAPHY

SEE BELOW

23. FUNDING SUPPORT FOR THIS STUDY

AHA Scientist Development Grant (PI: Pam Taub)

24. BIOLOGICAL MATERIALS TRANSFER AGREEMENT

N/A

25. INVESTIGATIONAL DRUG FACT SHEET AND IND/IDE HOLDER

N/A
26. IMPACT ON STAFF
None
27. CONFLICT OF INTEREST
Dr. Pam Taub (the principal investigator of this study) and her colleague, Dr. Francisco Villarreal, are both co-founders and minority shareholders in Epirium Bio (formerly Cardero Therapeutics) (a non-publicly traded company). Epirium Bio is developing drugs related to epicatechin for use in treatment of muscle disorders. This study is not sponsored by Epirium Bio and is fully funded by the American Heart Association. For questions, please contact the research coordinator at 858-246-2510 and they will connect the subjects to the appropriate staff at the Conflict of Interest Committee.
28. SUPPLEMENTAL INSTRUCTIONS FOR CANCER-RELATED STUDIES
N/A
29. OTHER APPROVALS/REGULATED MATERIALS
N/A
30. PROCEDURES FOR SURROGATE CONSENT AND/OR DECISIONAL CAPACITY ASSESSMENT
Only patients who are competent to provide consent will be enrolled in the study.

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