UCD0115B: An Open-label Extension Study of Purified Epicatechin to Improve Mitochondrial Function, Strength and Skeletal Muscle Exercise Response in Becker Muscular Dystrophy

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PROTOCOL SIGNATURE PAGE

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Original: January 20, 2015

Instructions to Investigators: Please sign and date this signature page, print your name, your title and the name of the facility in which the study will be conducted, and return a copy of this page to the Project Coordinator.

I confirm that I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practices, and the applicable laws and regulations of the country of the study site for which I am responsible, whichever provides the greater protection of the individual. I will accept the monitor's overseeing of the study.

Signature of Investigator	Date
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Signature of Representative of Study Sponsor	Date

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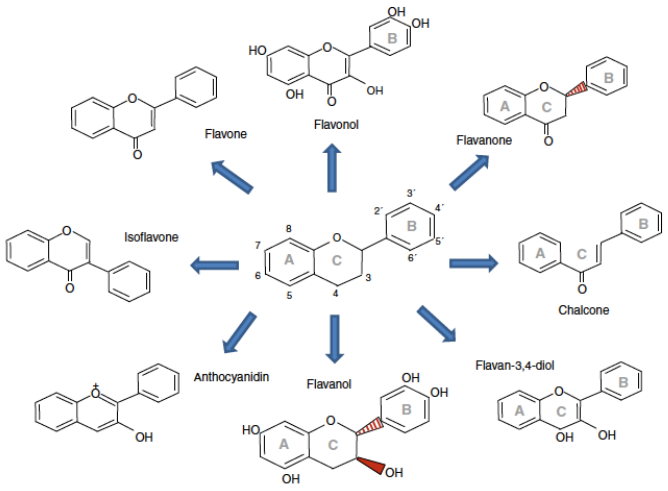
1 STUDY SUMMARY

1.1 Background

(-)-Epicatechin will be evaluated for the treatment of progressive muscle loss and impaired skeletal muscle function in Becker Muscular Dystrophy (BMD) patients. (-)-Epicatechin has been shown to improve muscle structure and function in animal and human studies by inducing muscle proteins, mitochondrial biogenesis and endogenous anti-oxidant mechanisms. (-)-Epicatechin and (-)-epicatechin-rich preparations have been well-tolerated with no significant safety issues, suggesting that (-)-epicatechin will be compatible with chronic administration. GMP grade (-)-Epicatechin will be provided by Cardero Therapeutics (CT).

(-)-Epicatechin (-)-Epicatechin is a naturally occurring compound found in many plants and fruits, including cocoa seeds, tea and grape[1, 2]. (-)-Epicatechin is a member of the flavanol subfamily of flavonoids, a chemically defined family of plant polyphenols (Figure 1) that have a basic structure of two aromatic rings (A and B) linked through three carbons that typically form an oxygenated heterocycle (C ring). The chemical characteristics of the C ring define the various subfamilies of flavonoids by providing different arrangements of hydroxy, methoxy, and glycosidic groups, and the bonding with other monomers[3].

Figure 1: Chemical structures of flavonoid families and (-)-Epicatechin



The majority of studies of (-)-epicatechin have utilized flavanol-rich cocoa preparations or concentrated tea extracts. Common commercial products that contain 60-90% cocoa can provide a total of ~12 mg of flavanols (monomers and multimers)/gram of chocolate. For example, 60% cocoa chocolate by Hershey will provide 0.84 mg/gram of (-)-epicatechin and 10.6 mg/gram of total flavanols (9.8 mg as multimers). (-)-Epicatechin, monomeric, isolated from tea, will be supplied by Cardero Therapeutics in gelatin capsules, each containing 25 mg (-)-epicatechin combined with excipients.

Many of today's medications are derived from plant extracts, including such commonly used medications as digoxin (foxglove), atropine (belladonna), codeine (poppy), irinotecan (dogwood) and taxol (pacific yew). Epicatechin is a flavonoid abundant in cacao that mimics the body's normal response to exercise training and that has demonstrated ability to promote increased mitochondrial growth and quality in multiple tissue types including muscle, effectively increasing the body's energy production capacity and reducing tissue oxidative stress. In populations where vigorous exercise is difficult or impossible and there is a high risk of secondary metabolic syndrome / insulin resistance such as individuals with muscular dystrophies, spinal cord injury, musculoskeletal or injury-induced mobility limitations, advanced heart disease, diabetes or even normal aging, the ability to rapidly produce an exercise training-like effect has wide-ranging benefits. These benefits include improved muscle mass, strength and endurance, insulin sensitivity, lipid profiles, and improved exercise capacity / physical activity. Pilot data from a trial of epicatechin in Becker muscular

dystrophy patients produced an unprecedented constellation of findings including increased muscle dystrophin, eutrophin, and follistatin levels and increased blood follistatin levels and decreased myostatin levels — all targets of current Duchenne muscular dystrophy treatment strategies. Treatment of individuals with Duchenne and Becker muscular dystrophies with epicatechin will yield a direct palliative effect, targeting multiple aspects of the complex cascade of events that if untreated ultimately leads to life-limiting loss of mobility, strength and cardiorespiratory function.

In a proof-of-concept study by our group, we demonstrated that epicatechin use in patients with Becker muscular dystrophy 1) improves muscle structure and function, 2) induces production or increased persistent content of muscle proteins including dystrophin, utrophin, sarcoglycans, and dysferlin, 3) induces mitochondrial biogenesis and 4) improves endogenous muscle growth, repair and regeneration mechanisms by increasing follistatin and decreasing myostatin levels. Epicatechin and epicatechin-rich preparations have been well-tolerated with no significant safety issues, suggesting that epicatechin will be compatible with chronic administration.

1.2 Study Purpose

This is a 48-week open-label extension of our initial proof-of-concept study (UCD0113) in patients with Becker muscular dystrophy who participated in the earlier trial. This single center study will enroll up to 8 adults who will receive the purified nutritional extract (-)-epicatechin 100mg/day orally for 8 weeks. After screening visits, participants will be enrolled in the study if they meet all inclusion criteria. They will be evaluated at screening, baseline, and weeks 4, 8, 12, 24, 16 and 48. The main criterion for success of the study will be presence of one or more biologic or strength and performance outcome measures (Aims 1 and 2 below) that yield a response magnitude that allows for sufficient power in a Phase II B study with a sample size of 30 individuals. Endpoints will include:

- *Efficacy Primary Biomarker:* Plasma biomarkers of follistatin, myostatin, follistatin:myostatin, BNP, NO and fasting insulin/glucose by ELISA and Western blot will be collected at baseline and weeks 12, 24, 36 and 48.
- *Efficacy Secondary Strength and Function:* Strength and exercise performance measures will be collected at baseline and weeks 12, 24, 36 and 48 for comparison with baseline measures.
- *Primary Safety:* A comprehensive physical/neurological exam, medical and adverse event history, hematology, blood chemistry and urinalysis safety profile will be collected at screening, baseline and weeks 4, 8, 12, 24, 36 and 48.
- *Exploratory Proteomics:* Plasma samples for proteomics analysis by mass spectrometry and Somalogic platform will be collected at baseline and weeks 24 and 48 for comparison with baseline measures.

1.3 Specific Aims:

Aim 1 (Efficacy Primary Endpoints): Evaluate the effect of epicatechin on circulating blood biomarkers of mitochondrial biogenesis and muscle regeneration in children with Duchenne muscular dystrophy. Individuals who receive oral epicatechin daily will show evidence of improved mitochondrial biogenesis. This will be reflected by plasma biomarkers associated with muscle growth and repair, vascular response to exercise, inflammation and membrane integrity. Increased number and quality of mitochondria will improve glucose metabolism and insulin response.

Aim 2 (Efficacy Secondary Endpoints): Evaluate the effect of epicatechin on exercise capacity, strength and function in children with Duchenne muscular dystrophy. Epicatechin will improve exercise capacity. Individuals who receive 24 to 48 weeks of oral epicatechin daily will show improved exercise and metabolic performance on the six-minute walk test and graded exercise cycle test that is associated with exercise “training-like” responses attributed to increases in mitochondrial biogenesis and muscle regeneration, and improvements in skeletal muscle perfusion. Individuals who receive 24 to 48 weeks of epicatechin will demonstrate improvements in strength and function as measured by clinical exercise testing parameters commonly used in muscular dystrophy clinical trials.

Aim 3 (Cardiac Efficacy Endpoints): Evaluate the effect of epicatechin on left ventricular strain and left ventricular ejection fraction. Individuals who receive 48 weeks of epicatechin 50-100mg daily will show improved measures of LV strain and ejection fraction compared to control subjects.

Aim 4 (Safety Primary Endpoints): Evaluate the safety and pharmacokinetic profiles of epicatechin in children with Duchenne muscular dystrophy. Epicatechin will demonstrate an acceptable safety profile. Assessments of safety will include a standard safety panel including hematologic, hepatologic, renal and metabolic profiles. Epicatechin administration to adults with BMD will yield pharmacokinetic profiles similar to those previously reported. Pharmacokinetic studies will include repeat assessments of trough, 2-hour post (peak) and 4-hour post epicatechin dose levels.

Aim 5 (Pilot Biomarker Endpoints): Perform pilot evaluation of disease- and epicatechin-specific circulating protein profiles as pilot biomarkers for monitoring treatment efficacy. Epicatechin stimulates follistatin expression in tissue and blood. In addition to its effects on muscle regeneration, follistatin is known to exert anti-inflammatory and anti-fibrotic effects via antagonism of activin and myostatin. Biomarker discovery studies will yield a panel of circulating proteins that are associated with treatment response.

2 BACKGROUND AND SIGNIFICANCE

2.1 The Dystrophinopathies: Duchenne and Muscular Dystrophy *(DBMD)*

Muscular dystrophies are a group of diverse genetic diseases featuring progressive muscle weakness, degradation of muscle fibers, and loss of function [4]. DBMD is an inherited spectrum disorder primarily seen in males, with an incidence of 1:3500-5000 live births [4]. Onset of symptoms can occur over a wide age range, with most patients diagnosed between 3 and 15 years of age [5, 6]. DBMD is characterized by progressive muscle loss (sarcopenia), with loss of strength, muscle injury, degeneration, atrophy, and eventually fibrosis and fatty replacement [7]. Patients with the more severe Duchenne (DMD) phenotype typically begin to lose ambulatory ability by 9-11 years of age and most are wheelchair dependent by 12-14 years. Most Becker (BMD) patients are more mildly affected and lose ambulation across a much wider age range from the late teenage years to late adulthood if at all. Both groups experience secondary complications of weakness including lung (e.g., breathing problems, infections) and heart (e.g., cardiomyopathy) complications that significantly impair mobility and participation and reduce lifespan[7, 8].

DBMD results from mutations in the gene encoding dystrophin, a key subsarcolemmal protein in the dystrophin-associated protein complex (DAPC) at the muscle cell membrane [9]. The DAPC links the muscle cytoskeleton with the extracellular matrix and appears to play a role in muscle stabilization during contraction as well as nNOS signaling [10, 11]. Loss of dystrophin destabilizes the DAPC and results in abnormal muscle membrane permeability, detectable as large elevations in plasma creatine kinase at birth and before the appearance of physical symptoms [5]. BMD is attributed to quantitative or qualitative decrements in dystrophin expression, while patients with DMD experience complete or near-complete loss of dystrophin.

2.2 Epicatechin directly targets multiple points in DBMD pathophysiology

The process of progressive strength loss in individuals with dystrophinopathies is due to a complex pathophysiologic cascade that occurs as a secondary effect of the loss of the dystrophin protein. Progressive damage to muscle tissue occurs due to changes in the structure of the contractile apparatus due to partial or complete loss of the dystrophin associated protein complex (DAPC), and results in secondary damage to and depletion of mitochondria. The mitochondrial injury itself is attributed to oxidation injury, calcium accumulation and excessive metabolic demands of regenerating muscle, where cellular energy demands are not sufficient to compensate. Alteration of expression profiles associated with improved oxidative phosphorylation in normal exercise response are decreased in DMD and reduce effectiveness of tissue remodeling [12, 13]. Activity or exercise-induced mitochondrial upregulation is also associated with improvements in insulin resistance and reduced incidence of diabetes, and decreases in mitochondrial capacity seen in dystrophinopathy patients are also seen in diabetic patients, suggesting that reduced activity or capacity in these pathways more accurately reflects physical activity status rather than any specific disease process. Thus, it is expected that drugs targeting mitochondrial biogenesis will have wide-ranging applications outside of the dystrophinopathies, and may also include groups such as patients

with diabetes and obesity, age-related muscle wasting (sarcopenia), and sarcopenias that occur secondary to other conditions such as spinal cord injury and stroke

Epicatechins represent a novel small molecule approach to intervention directly upregulating this mitochondrial pathway. Treatment with epicatechins increases mitochondrial synthesis of ATP in response to metabolic demand within 48 hours, and concurrently activates the transcription pathway for muscle growth and regeneration by upregulating the protein PGC1a, which in turn upregulates follistatin, which is a key regulator of the growth antagonist myostatin. This has been demonstrated in neonatal mice, where PGC1a over expression resulted in increased expression of key muscle proteins including utrophin and type-1 myosin heavy chain as well as key mitochondrial proteins [14]. Overexpression of PGC1a also drives oxidative gene expression, aiding damaged mitochondria and supporting ATP production [15-20]. Selsby et al demonstrated that the increase in production of muscle proteins secondary to PGC1a overexpression increased Type 1 muscle fiber count, improved resistance to eccentric contraction-induced injury and fatigue and reduced evidence of muscle tissue necrosis in the *mdx* model of dystrophinopathies, and provides evidence that the approach improves dystrophic muscle resistance to structural damage secondary to activity and exercise [14].

Further evidence suggests that NF-kB, a protein which contributes to healthy tissue remodeling and adaptation in response to exercise, paradoxically reduces activation of PGC1a pathways in diseased conditions such as dystrophinopathies, diabetes and cachexia, contributing to increased metabolic and oxidative stress to muscle tissue and subsequent loss of mitochondria, increased inflammation, and tissue degeneration [21].

In addition to decreased resistance to mechanical stressors, patients with dystrophinopathies also under express nitric oxide (NO) and show evidence of a subsequent lack of normal vascular response to exercise[22]. This reduces oxygen transport capacity to muscles during exercise, creating an environment where the tissue is chronically oxygen-starved during activity. Oxygen depravation leads to increased oxidative stress as the remaining mitochondria are forced to utilize relatively greater proportions of anaerobic mechanisms to create energy for the muscle tissue. The by products of this anaerobic process include damaging pro-inflammatory reactive oxygen species (ROS) that have been shown to trigger chronic activation of the NF-kB pathway discussed above.

The combination of reduced resistance to mechanical stress and reduced oxygen supply and resulting chronic inflammation creates a “double hit” that promotes a vicious cycle of tissue damage, inflammation and impaired remodeling that ultimately results in gradual and eventually almost complete replacement of muscle with non-functional fibrotic and fatty tissue.

In the absence of genetic repair, there is a great unmet clinical need for palliative therapies that can slow the progressive loss of muscle function due to secondary damage and/or improve skeletal muscle function. The epicatechin signaling pathway represents a *direct* intervention that addresses many of the defects that occur secondary to the loss of dystrophin that are currently under investigation. Treatment with the drug directly increases expression of follistatin resulting in reduction in production of myostatin and improved muscle growth and regeneration. Indirect immune-mediated reductions in myostatin using targeted antibodies has become a key therapeutic approach in dystrophinopathies [23]. Epicatechin use also results in upregulation of PGC1a which increases the number and quality of mitochondria in muscle tissue that leads to reduced oxidative stress, improved muscle function and improved insulin resistance. Indirect approaches to reduce oxidative stress using CoenzymeQ10 and idebenone are currently in development in DMD and have shown positive results [24, 25]. Indirect approaches to reducing inflammation and tissue remodeling that occur secondary to oxidative stress include ongoing study of anti-fibrotic drugs such as pentoxifylline and halofuginon [26-28]. Treatment with epicatechin directly results in increased NO production, improves vascular response to exercise and reduces tissue hypoxia and oxidative stress. Studies of the ability of PDE5 inhibitors (sildenafil, tadalafil) to block downstream degradation of of NO-derived vasodilators is currently being explored in DMD patients to improve oxygen supply to muscle tissue[29]. A summary of published and unpublished preclinical and clinical data supporting the evaluation of epicatechin in DBMD is presented in **Table 1**.

Table 1: Effects of epicatechin on progressive muscle loss parameters.

Progression of MD due to:	Epicatechin Effects
Loss of Dystroglycan Proteins	Stimulated the expression of multiple components of the dystroglycan protein assembly in mice and humans, including compensatory expression in a MD mouse model
Mitochondrial Depletion	Increased expression of transcription factor PGC1a and stimulated mitochondrial biogenesis, increased expression of all electron transport complex proteins and increased mitochondrial density and mitochondria cristae density in mouse and human muscle
nNOS Deficiency	In a pilot clinical trial in patients with diabetes and heart failure, epicatechin-treated patients showed a statistically significant increase in quadriceps muscle nNOS expression
Oxidative injury	Increased the expression of endogenous anti-oxidant enzymes superoxide dismutase and catalase and increased total thiols in skeletal muscle of aged patients with diabetes and heart failure
Muscle Degeneration	Reduced creatine kinase activity (plasma marker for muscle membrane injury); improved histological appearance in sarcomere morphology in aged patients with diabetes and heart failure
Impaired Muscle Regeneration	Increased markers of muscle regeneration in skeletal muscle of mdx mice and aged patients with diabetes and heart failure
Muscle Weakness	Improved muscle strength in mdx mouse model of MD and physical activity in the delta Sarcoglycan KO mouse model of limb girdle dystrophy; increased grip strength in middle-aged human volunteers
Inflammation And Fibrosis	Prevented the myocardial fibrosis associated with the delta-sarcoglycan KO mouse model of MD

It must be stressed that these muscle-related therapeutic activities are unique to epicatechin and do not represent general flavonoid properties, as other flavonoids such as quercetin fail to exert similar effects. Epicatechin biology is chirally specific, as its diastereomer, catechin, is not only inactive in stimulating mitochondrial biogenesis, it antagonizes epicatechin's effects, possibly due to steric competition at a binding site. Nor is the effect due to anti-oxidation, as catechin and quercetin are anti-oxidants equal in potency to epicatechin but are inactive with respect to mitochondria.

3 EPICATECHIN OVERVIEW

3.1 Pharmacokinetics / Pharmacodynamics

3.1.1 (-)-Epicatechin

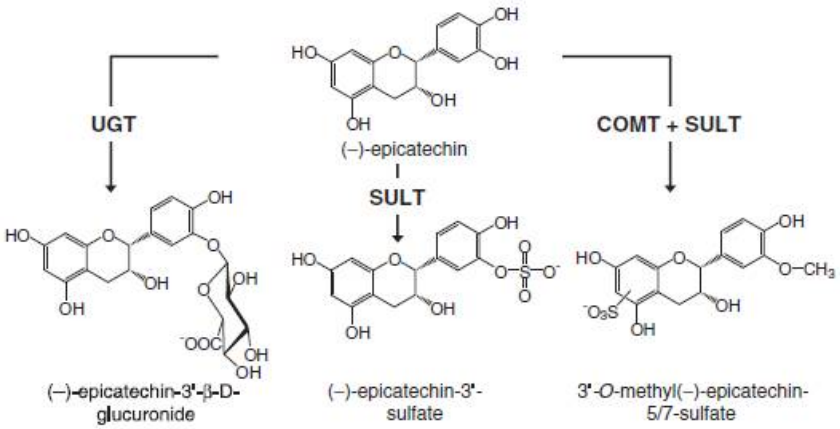
(-)-Epicatechin is orally bioavailable, as the consumption of (-)-epicatechin or cocoa products can result in pharmacologically relevant levels of (-)-epicatechin and its metabolites in blood. In addition to the literature reviewed below, the results of a recent human pharmacokinetic study are presented in Section 6.3.
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 methylated [30, 31], allowing negligible amounts of native (-)-epicatechin in the mesenteric circulation.

In the liver, further glucuronidation, methylation, and sulfation can take place [30, 32, 33]. Several studies have determined the presence of these conjugates in the plasma and urine of rodents and humans [33-38], as well as in rat bile [32] and brain [39]. In plasma, (-)-epicatechin is present almost exclusively as conjugated metabolites[37, 38].

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 [34, 37-46]. The most abundant (-)-epicatechin metabolites detected in plasma after dark chocolate ingestion were (-)-epicatechin-3'-β-D-glucur- onide,(-)-epicatechin3'-sulfate,and 3'-O-methyl-(-)-epicatechin-5-sulfate[45]. A schematic representation of these metabolites is shown in Figure 2. In addition, significant levels of 3'-O-methyl-(-)- epicatechin sulfates substituted in the 4' and 7 positions were identified [45].

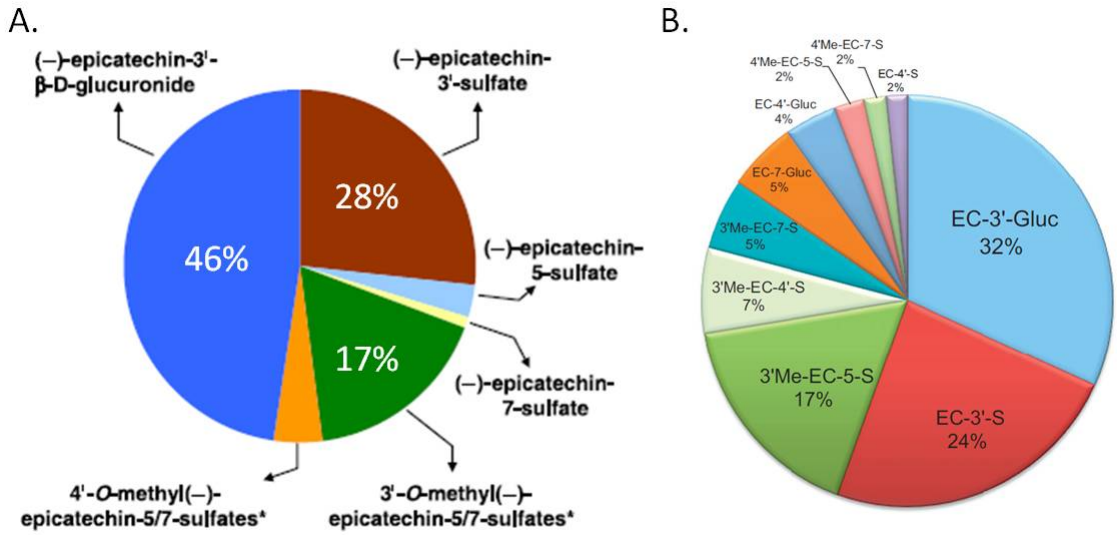
Relative amounts of (-)-epicatechin metabolites identified in plasma in two separate studies are shown in Figure 3.

Figure 2: Major (-)-epicatechin metabolites in plasma.



Schematic representation of the primary structurally-related (-)-epicatechin metabolites found in plasma after injestion of (-)-epicatechin-containing test beverage [38]. UGT, UDP-glucuronosyltransferase; SULT, sulfotransferase; COMT, catechol O-methyltransferase.

Figure 3: (-)-Epicatechin metabolite profile in plasma



A. *(-)-Epicatechin metabolite profile in plasma 2 hours after ingestion of 1.8 mg/kg body weight (-)-epicatechin [38]. Unmodified (-)-epicatechin represented <0.5%.* ***B.*** *Profile of (-)-epicatechin metabolites in plasma after ingestion of 100g of dark chocolate ((79 mg (-)-epicatechin)) [37]. Data are expressed as the percentage of total plasma AUC0–24 h. Unmodified (-)-epicatechin not detected. EC-3'-Gluc, (-)-epicatechin-3'-β-D-glucuronide; EC-4'-Gluc, (-)-epicatechin-4'- β-D-glucuronide; EC-7-Gluc,(-)-epicatechin-7-β-D-glucuronide; EC-3'-S, (-)-epicatechin-3'-sulfate; 3'Me-EC-5-S, 3'-O-methyl-(-)-epicatechin 5-sulfate; 3'Me-EC-4'-S, 3'-O-methyl-(-)-epicatechin 4'-sulfate; 3'Me-EC-7-S, 3--O-methyl- (-)-epicatechin 7-sulfate; and 4'Me-EC-5-S, 4'-O-methyl-(-)-epicatechin 5-sulfate.*

The T_{max} (time to maximum plasma concentration) of (-)-epicatechin and most of its metabolites is 1-2 hours, elimination half-time ($t_{1/2}$) from plasma is ~2-2.5 hours, and over 90% of the urinary excretion of these compounds was complete by 8 hours[38, 46, 47]. Oral bioavailability (peak plasma concentration/mg ingested) for (-)-epicatechin in cocoa/chocolate is ~10-20 nM/mg injested (Table 2). Assuming ~3L plasma in a 75 kg man, oral bioavailability of (-)-epicatechin (% ingested dose in plasma at Cmax, total epicatechin and metabolites), can be estimated as 1-2%. A value of 1.1% was reported for a 45 mg (-)-epicatechin dose in a green tea catechin mixture [47].

Table 2: Selected human oral bioavailability studies of (-)-epicatechin

Reference	Test Article	(-)-Epicatechin (mg)	Peak (-)-Epicatechin Species in Plasma (nM)	Peak (-)-Epicatechin Exposure (nM/mg)
Loke, W.M., et al., 2008 [48]	(-)-Epicatechin	200	3570	17.85
Ottaviani, J.I., et al., 2011 [43]	(-)-epicatechin added to cocoa vehicle	125 (mean)	~900	~7.2
Ottaviani, J.I., et al., 2012 [38]	**Cocoa drink**	**135 (mean)**	1245	9.3
Actis-Goretta, L., et al., 2012 [37]	**Dark Chocolate**	**79**	873	11.1
Donovan, J.L., et al., 2006 [49]	**Cocoa drink**	**55**	630	11.5
Schroeter, H., et al., 2006 [42]	**Cocoa flavanols:** 917 mg	174	~1950	~11.2
Baba, S., et al., 2000 [35]	**Dark chocolate**	**220**	4770	21.7

Oral doses of (-)-epicatechin between 25 and 200 mg have been shown to be pharmacologically relevant. A 200 mg oral dose increased measures of plasma NO and decreased the vasoconstrictor endothelin-1 [48], while administration of an ~75 mg dose (1 mg/kg body weight) resulted in significant improvement in vascular function (FMD) [42]. A recent meta-analysis of clinical data reported that an intake of 25 mg (-)-epicatechin per day was associated with significant reductions in SBP and DBP [50].

4 PRELIMINARY STUDIES

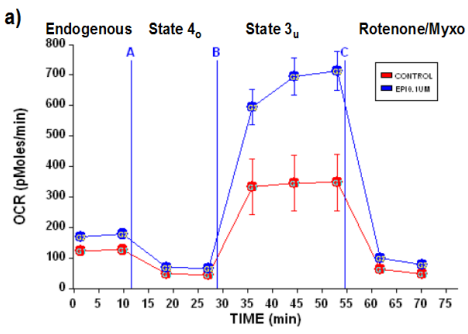
4.1 Preclinical Data

Collaborators of Cardero Therapeutics at the University of California, San Diego (UCSD) have conducted cell culture and animal studies examining the ability of (-)-epicatechin to induce mitochondrial biogenesis and SkM protein synthesis, resulting in improved muscle structure and function.

4.1.1 (-)-Epicatechin Increases Mitchondrial Respiration and Biogenesis in Cultured Human Skeletal Muscle Cells (unpublished data)

The effect of (-)-epicatechin on mitochondrial respiration, biogenesis and structure in human skeletal muscle cells *in vitro* was examined. Human skeletal muscle cells from a healthy subject were treated with 0.1 µM of (-)-epicatechin (in cell culture) for 48 hours. The treated muscle cells manifested a markedly enhanced capacity for oxidative phosphorylation (Figure 4). The ability of epicatechin to stimulate the capacity for oxidative phosphorylation in human muscle fibers treated with epicatechin allows the muscle cell to substantially increase its rate of oxidative phosphorylation in response to the metabolic demand placed on the cells by FCCP, which decreases the mitochondrial membrane potential. Importantly for safety reasons, neither endogenous nor resting oxidative phosphorylation in muscle cells is affected.

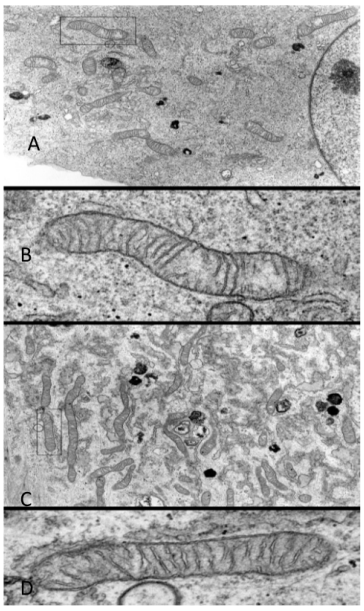
Figure 4: (-)-Epicatechin enhances mitochondrial respiration



. Treatment of Primary Cultures of Human Skeletal Muscle Myocytes with Epicatechin Enhances Maximal Rates of Mitochondrial Respiration. Effects of treatment of cultured myocytes for 48 hours with epicatechin on mitochondrial respiratory rates was monitored using the Seahorse XF24. Cells were plated at 30,000/well and treated with the indicated concentration of epicatechin in normal culture medium. Respiration (O_2 consumption rate, OCR) of the intact cells was measured in unbuffered DMEM containing 10 mM glucose, 10 mM pyruvate, and 2 mM glutamine. In **a)** endogenous respiration was measured on cells from subject 1, followed by state 4_o (resting) respiration with the addition of 1 uM oligomycin (after 'A'), and then maximal rates (state 3_u) were measured after the addition of 300 nM FCCP, a chemical uncoupler (after 'B'). Rotenone plus myxothiazol (100 nM each) was then added to assess non-mitochondrial O_2 consumption (after 'C'). In b) a dose response to epicatechin for 48 hours was performed with cells from subject 2, and maximal rates of respiration were measured with addition of 300 nM FCCP.

Treated cells were then examined using electron microscopy (Fig 5). The electron micrographs illustrate two effects. One is the marked increase in mitochondrial number. Even more unusual is the increased number of cristae per mitochondrion, suggestive of the potential for increased ATP synthesis per mitochondrion. To our knowledge, cristae density within a mitochondrion has not previously been demonstrated to be acutely modulatable before. Mitochondrial biogenesis can also be blunted by the use of eNOS inhibitors, supporting a role for the NO system in the mechanism of action for (-)-epicatechin's effects.

Figure 5: (-)-Epicatechin increases mitochondrial number and cristae density in human skeletal muscle cells (unpublished data)

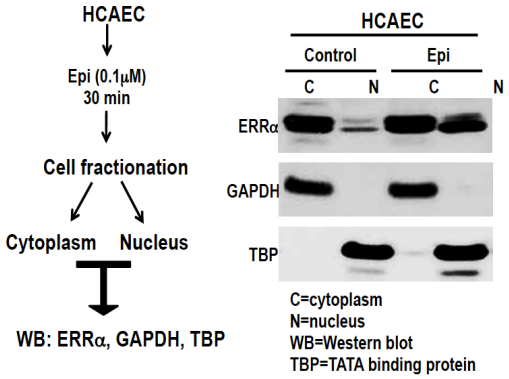


Electron microscopy of human skeletal muscle cells from healthy human subjects treated with 0.1 uM of (-)-epicatechin (in cell culture) for 48 hours are shown in panels C and D; cells from the same subject without treatment are shown in A and B. Based on blinded independent analysis of cristae/mitochondrial membrane ratio the (-)-epicatechin treated cells (1.33 ±.0 34 vs. control 0.88 ± 0.49) had statistically significant (p=0.03) more cristae membrane where the oxphos complexes are located, suggesting (-)-epicatechin-treated cells have a greater capability for ATP generation. In addition to increases in cristae membrane, treated cells also had an increased number of mitochondria.

4.1.2 (-)-Epicatechin Induces Mitochondrial Biogenesis Pathway in Human Coronary Artery Endothelial Cells (unpublished data)

Recent unpublished experiments established that (-)-epicatechin initiates mitochondrial replication via the activation of two co-factors that participate in the classical transcription pathway for mitochondrial biogenesis: The Estrogen Related Receptor (ERR) pathway and the activation of PGC1α. The ERR pathway comprises an orphan nuclear receptor complex consisting of 3 subunits, α, β and γ. When exposed to the unknown ligand, they trimerize in the cytoplasm and form a complex with PGC1α, and subsequently localize to the nucleus, where they initiate the transcription pathway for mitochondrial biogenesis [51]. Nuclear localization of the α subunit is accepted as an indicator of ERR activation. Human coronary artery endothelial cells (HCAEC) exposed to (-)-epicatechin demonstrated localization of ERRα to the nucleus within 30 minutes (Fig 6).

Figure 6: (-)-Epicatechin induces estrogen-related receptor alpha translocation (activation) into the nucleus of HCAEC



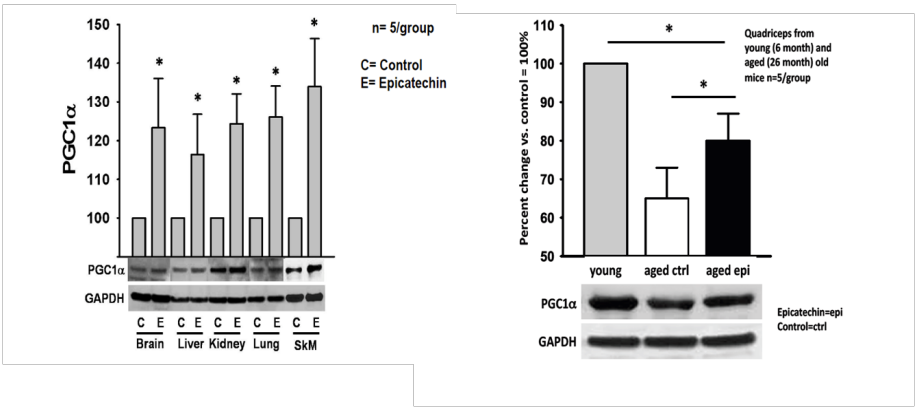
4.1.3 (-)-Epicatechin Induces Expression of Mitochondrial Biogenesis Factor PGC-1α in Aged Mice (unpublished data)

The peroxisome proliferator-activated receptor gamma (PPARγ) coactivator-1α (PGC-1α) is a transcriptional coactivator of nuclear receptors and other transcriptional factors that can enhance multiple aspects of cellular energy metabolism, including mitochondrial biogenesis and angiogenesis [52, 53]. Expression of PGC-1α in cultured mammalian cells or specific tissues of transgenic mice increases number and mass of mitochondria together with a strong enhancement of cellular respiratory capacity[17], suggesting that PGC-1α would be expected to enhance mitochondrial biogenesis and promote muscle repair and regeneration. The effects of (-)-epicatechin on PGC-1α levels were examined in young (6 mo), and old (26 mo) mice. Senile mice treated with epicatechin for two weeks exhibited increased expression of PGC-1α across all tissues evaluated (Fig 7A). These animals also demonstrated correlative mitochondrial biogenesis (not shown). When PGC-1α level of expression was compared in the senile mice to young mice, they were markedly diminished, and stimulated by epicatechin treatment in as little as two weeks (Fig 7B).

Figure 7: (-)-Epicatechin increases PGC1-alpha in aged mice.

A. Effects of epicatechin on PGC-1α in 26 month old mice tissues

B. PGC-1α in young and old (+/- Epi) mice quadriceps tissues



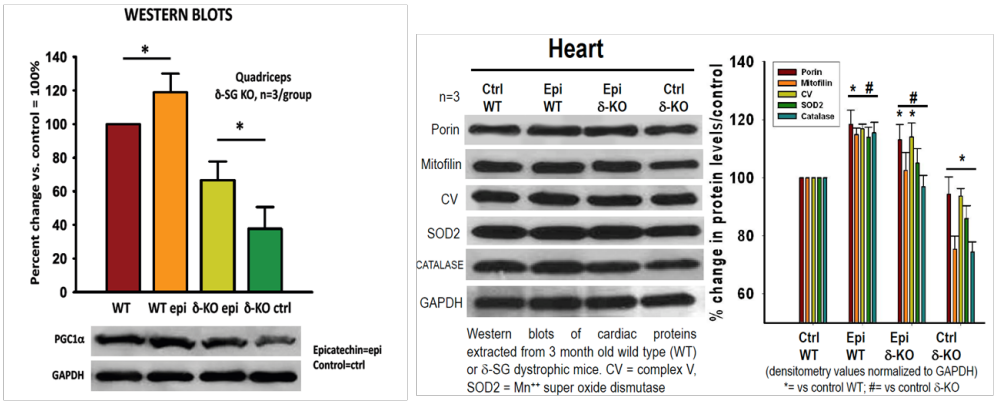
4.1.4 (-)-Epicatechin Induces Expression of Mitochondrial Biogenesis Factor PGC-1α and Mitochondrial Proteins in δ-sarcoglycan KO Mice (unpublished data)

The effects of (-)-epicatechin on PGC-1α levels were examined in the δ-sarcoglycan KO mouse model of MD. 3 month old WT and δ-sarcoglycan mice were treated with (-)-epicatechin or water for 1 month. In the δ-sarcoglycan KO mice, the loss of PGC1α is striking, as is its stimulation with 1 month of epicatechin treatment (Fig 8A). The change in PGC1α correlated with loss and regain of mitochondrial density (Fig 8B). The KO mice treated with water exhibited a significant depletion of mitochondrial proteins – enzymes and structural proteins, during a phase where they developed fibrotic cardiomyopathy. Epicatechin restored mitochondrial protein levels to normal levels, suggesting that (-)-epicatechin induced PGC-1a-mediated mitochondrial biogenesis.

Figure 8: (-)-Epicatechin increases PGC1-alpha and mitochondrial proteins in gamma-sarcoglycan knock out mice.

A. Effects of epicatechin on PGC-1α in WT and δ-sarcoglycan KO mice

B. Mitochondrial proteins in WT and δ-sarcoglycan KO mice

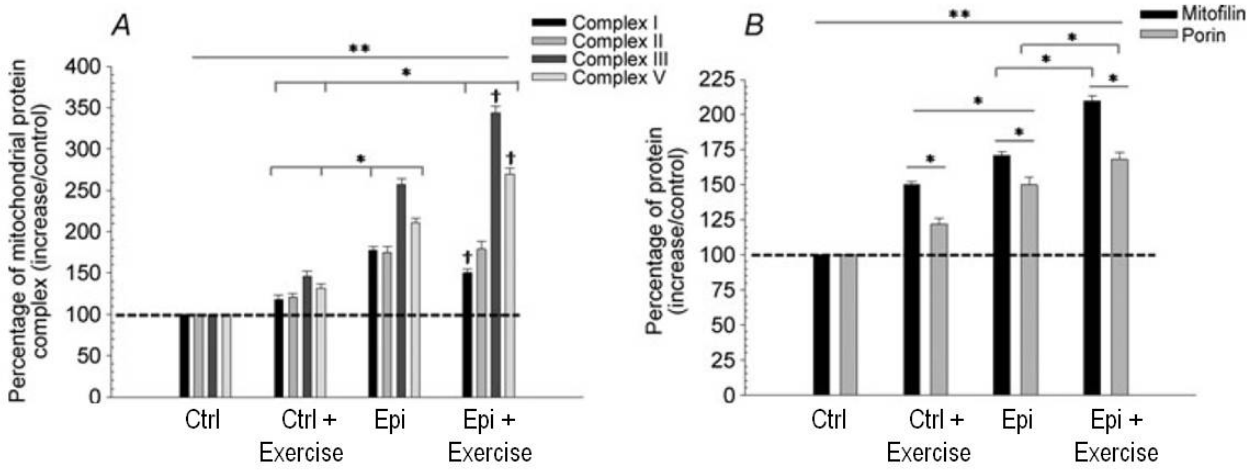


4.1.5 (-)-Epicatechin Increases I*n Vivo* Mitchondrial Biogenesis and Muscle Function in Aged 1 Year Old Mice

To test for the *in vivo* effects of (-)-epicatechin on muscle performance and indicators of mitochondrial structure (porin, mitofilin) and biogenesis (Tfam), studies were conducted in mice to compare the effects of (-)-epicatechin +/- daily exercise with vehicle +/- daily exercise[54]. Aged one year old male mice were subjected to two weeks of (-)-epicatechin treatment (1 mg/kg BID, dissolved in water) by oral gavage. Significant increases in treadmill performance (≈50%) and enhanced in situ muscle fatigue resistance

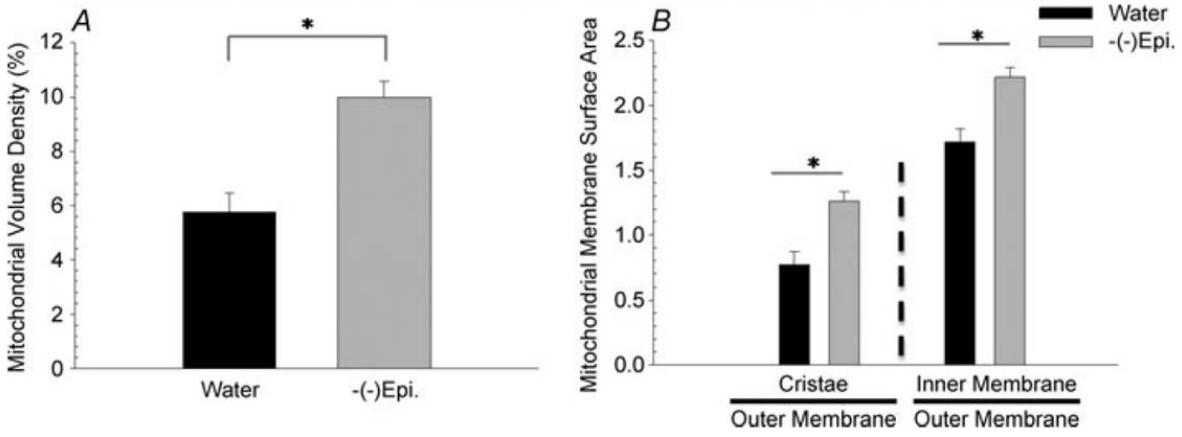
(≈30%) were observed with (-)-epicatechin. Components of oxidative phosphorylation complexes, mitofilin, porin, nNOS, and Tfam as well as mitochondrial volume and cristae abundance were significantly higher with (-)-epicatechin treatment for hindlimb and cardiac muscles than exercise alone (Figures 9 and 10). In addition, there were significant increases in skeletal muscle capillarity. The combination of (-)-epicatechin and exercise resulted in further increases in oxidative phosphorylation complexes proteins, mitofilin, porin, and capillarity than (-)-epicatechin alone. These findings indicate that (-)-epicatechin alone or in combination with exercise induces an integrated response that includes structural and metabolic changes in skeletal and cardiac muscles resulting in greater endurance capacity.

Figure 9: (-)-Epicatechin increases mitochondrial proteins in 1-year old mice.



Effect of (-)-epicatechin and exercise on A) mitochondrial oxidative phosphorylation complexes and B) mitochondrial membrane proteins. Ctrl = water only, Epi=1 mg/kg BID. Exercise = 30 minutes of treadmill exercise 5 times per week.

Figure 10: (-)-Epicatechin increases mitochondrial volume density and mitochondrial membrane surface area in 1-year old mice.



Electron microscopic examination of plantaris muscle to calculate A) mitochondrial volume density (% of cytoplasm occupied by mitochondria) and B) mitochondrial membrane surface area for cristae and inner membrane, normalized to outer membrane area.

These results are consistent with a recent study by Hutteman et al which described the ability of (-)-epicatechin to maintain exercise-induced improved capillarity and mitochondrial capacity in mice after discontinuation of exercise training, in part by induction of mitochondrial complex proteins [55].
In a follow up study the capacity of (-)-epicatechin treatment to stimulate myocardial angiogenesis was examined in the same animals. Results indicate that exercise training or (-)-epicatechin significantly stimulated myocardial angiogenesis by 30-35% above control levels (as judged by biochemical and histological measures) whereas the use of both lead to further significant increases (to ~50%). Exercise training effects were associated with significant increases in protein levels and/or activation (i.e. phosphorylation) of canonical angiogenesis pathway associated events [vascular endothelial growth factor

(VEGF), eNOS, NO and cGMP]. In most cases, (-)-epicatechin generated comparable degrees of stimulation of these pathways. The use of combined treatment led from incremental to additive outcomes in these signaling pathway endpoints.

4.1.6 (-)-Epicatechin Increases Mitchondrial Biogenesis and Improves Myocardial Function in Rats

Results from a series of studies in a rat myocardial infarction model suggests that (-)-epicatechin may facilitate preservation of myocardial function via preservation of mitochondrial structure and function. In the first study [56] (-)-epicatechin (1 mg/kg/day) or water (control) pretreatment was administered daily via oral gavage to male rats for 2 or 10 days. Ischemia was induced via a 45-min coronary occlusion. Reperfusion was allowed until 48 h or 3 wk while treatment continued. With 2 days of treatment, no reductions in myocardial infarct (MI) size occurred. After 10 days, a significant ~50% reduction in MI size occurred in the (-)-epicatechin-treated animals. (-)-Epicatechin rats demonstrated no significant changes in hemodynamics. Tissue oxidative stress was reduced significantly with (-)-epicatechin treatment. Matrix metalloproteinase-9 activity demonstrated limited increases in the infarct region with (-)-epicatechin. By 3 wk, a significant 32% reduction in MI size was observed with treatment, accompanied with sustained hemodynamics and preserved chamber morphometry.

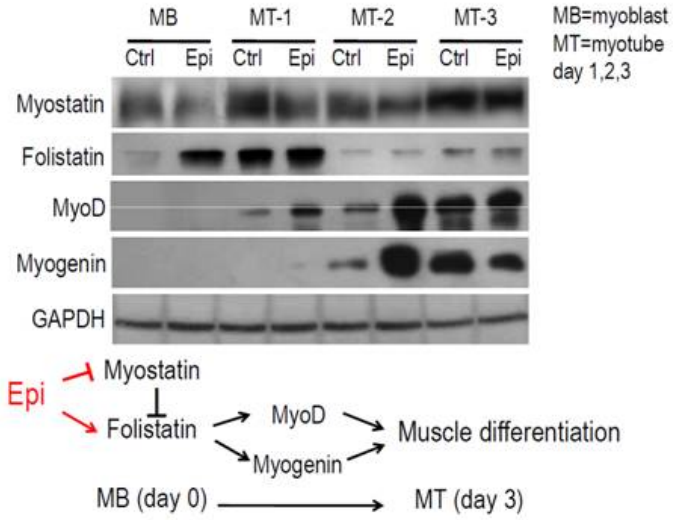
A subsequent study mirroring the one noted above was performed but using a more severe modality of myocardial injury which over time can trigger the development of heart failure (permanent coronary occlusion) [57]. Results from this study also indicated significant reductions in MI size by (-)-epicatechin (1 mg/kg/day) early after occlusion (48 h) that were sustained over time (3 weeks). Treatment did not alter hemodynamics. These effects were accompanied by the significant long-term (3 week) preservation of myocardial structure and function.

An additional study was implemented to test the potential of (-)-epicatechin to exert cardioprotection during I/R via modulation of mitochondrial function [56]. Ischemia was induced in rats via a 45 min occlusion, followed by reperfusion for 1 h, 48 h, or 3 weeks (wk). (-)-Epicatechin (10 mg/kg) was administered IV 15 min prior to reperfusion for the single dose group and again 12 h later for the double dose group. Controls received water. A single dose of (-)-epicatechin significantly reduced infarct size by 27% and 28% at 48h and 3 wk, respectively, compared to controls. Double dosing further decreased infarct size at 48 h by 80%, which was sustained at 3 wk (52% reduction). In order to assess if (-)-epicatechin-induced cardioprotection was mediated by protection of mitochondrial function, mitochondria were isolated from the left ventricle of sham, I/R, and I/R + (-)-epicatechin animals 1 h after ischemia. I/R animals had a significant decrease in mitochondrial O2 consumption, significant increase in mitochondrial Ca2+ levels, and decreased ATP and NADH pools. (-)-Epicatechin protected against these changes and had levels similar to sham animals. Taken together, results suggest that (-)-epicatechin preserves myocardial bioenergetics which likely underlies the cardioprotection observed.

4.1.7 (-)-Epicatechin Modulates the Synthesis of Muscle Growth and Differentiation Proteins in Cultured Cells and Mice (Aged and MD)(unpublished data)

The ability of (-)-epicatechin to effect markers of muscle growth and differentiation were assessed in cultured C2C12 myoblasts, 6 and 26 month old mice and mdx mice (MD model).
Muscle Regulatory Protein Expression in C2C12 Cells C2C12 cells were cultured +/- (-)-epicatechin in the presence of 1% horse serum, which induces differentiation (myoblast to myotube transition). Figure 11 depicts the three day time course of differentiation (by Western blots). (-)-Epicatechin treatment accelerated and increased protein levels of the muscle growth factor follistatin and those of differentiation (myogenin, MyoD), while decreasing levels of the inhibitor myostatin, suggesting that (-)-epicatechin upregulates muscle differentiation.

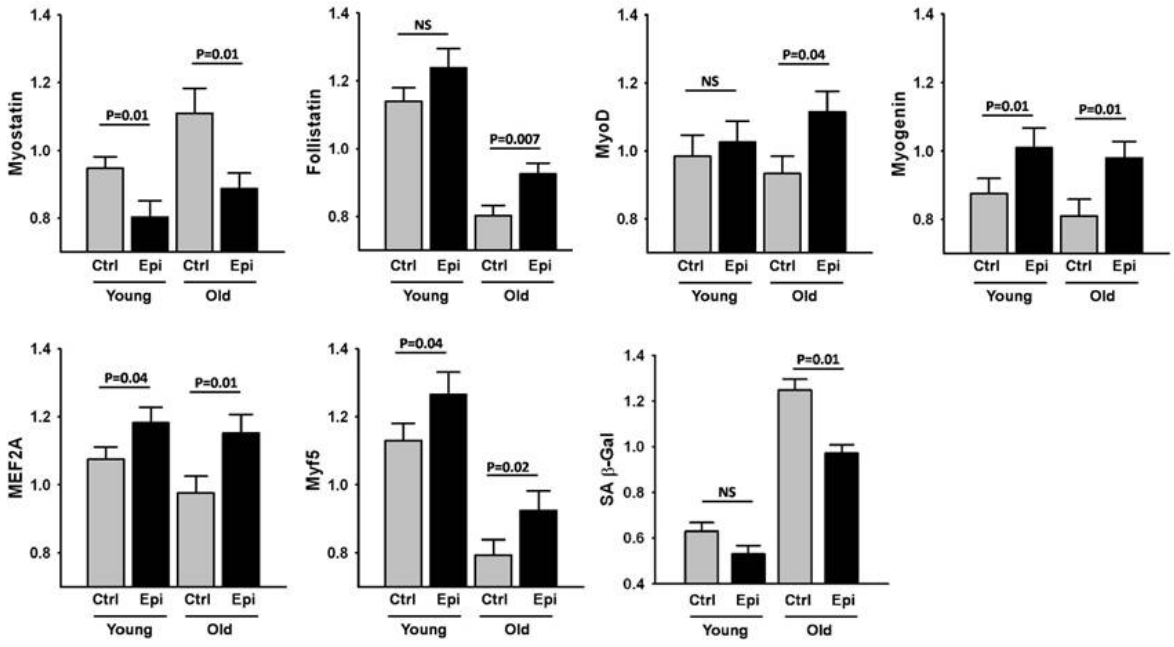
Figure 11: Muscle differentiation in time course in C2C12 cells +/- (-)-epicatechin.



4.1.8 Muscle Regulatory Protein Expression in 6 and 26 month old Mice

The expression of muscle growth, differentiation and senescence proteins in quadriceps SkM biopsies from 6 and 26 month old mice, either untreated (control) or treated with 1 mg/kg (-)-epicatechin for 15 days, was quantified by Westerns (Figure 12). There were clear age-related changes in protein expression, with notable increases in SkM myostatin and decreases in follistatin protein levels. Modest decreases were observed in MEF2, MyoD and myogenin with larger differences noted for Myf5. As expected, large increases were observed in senescence-associated β-galactosidase: (SA-β-Gal), a cell senescence marker, in older animals. As in the C2C12 cells, (-)-epicatechin treatment decreased myostatin while increasing the levels of differentiation-promoting factors, with three of the factors (MyoD, MEF2 and myogenin) reaching levels in the old mice that were similar or greater than those seen in young control mice.

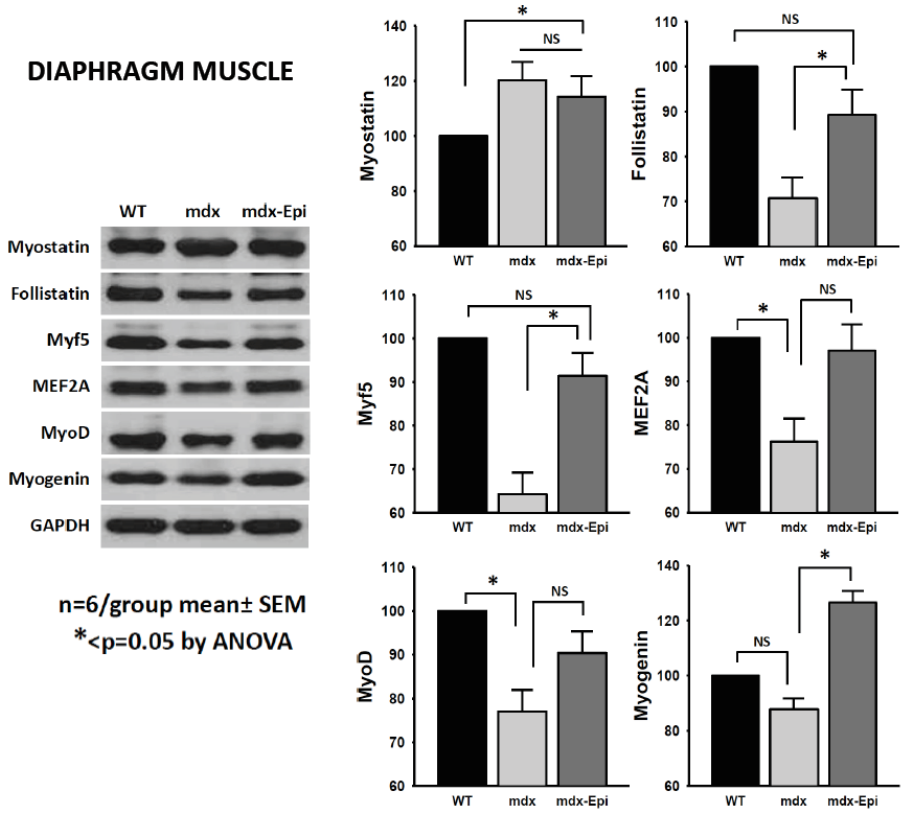
Figure 12: Muscle regulatory protein expression in 6 and 26 month old mice +/- (-)-Epicatechin



4.1.8.1 Muscle Regulatory Protein Expression in mdx Mouse Model of MD

Figure 13 depicts representative images obtained from Western blots performed on diaphragm muscle samples from mdx mice treated for month with (-)-epicatechin starting at 12-16 weeks of age. Muscle samples were probed to evaluate changes in protein levels for the muscle growth modulators, myostatin and follistatin. As can be observed in the upper panels, in mdx mice there is a significant upregulation of myostatin that did not improve with treatment. In contrast, follistatin levels in the diaphragm were significantly reduced in water treated mdx mice and recovered with (-)-epicatechin. Follistatin is a trophic muscle hormone well known to promote muscle regeneration and well as inhibit fibrosis and inflammation. Changes in recognized regulators of muscle differentiation were evaluated (Myf5, MEF2A, MyoD and myogenin). As can be observed, with the exception of MyoD where (-)-epicatechin treatment did not fully recover protein levels, all other modulators were restored to wild type (WT) levels with treatment. Similar results were obtained in an analysis of the mdx gastrocnemius muscle (data not shown).

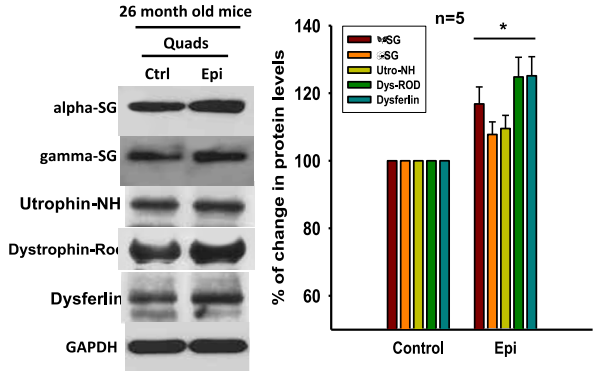
Figure 13: (-)-Epicatechin modulates muscle regulatory proteins in diaphragm muscle in *MDX* mice.



4.1.9 (-)-Epicatechin Stimulates the Expression of MD-Relevant Muscle Structural Proteins in Aged and MD Mice (unpublished data)

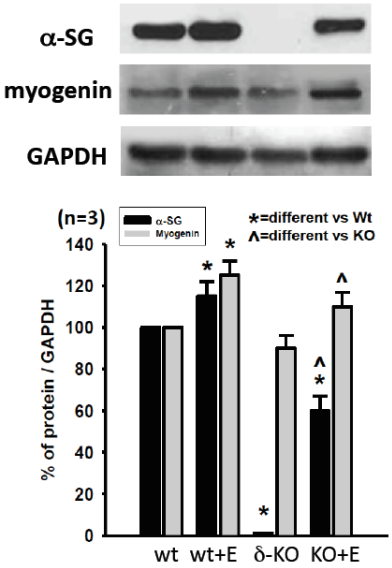
The effects of (-)-epicatechin in animals experiencing progressive muscle loss was examined in aged normal mice (model of sarcopenia) and δ-sarcoglycan KO mice (model of limb-girdle muscular dystrophy). In examining muscle loss associated with age, 26 month old mice, within 6 months of the end of their natural lifespan, were treated with (-)-epicatechin (1mg/kg) for just two weeks. All mice demonstrated increases in dystrophin, the sarcoglycans, and desferlin when compared by Western blot to age-matched controls (Fig 14).

Figure 14: (-)-Epicatechin increases DAPC proteins in middle aged mice.



The effect of epicatechin's stimulation of the dystrophin protein complex was also examined in the δ-sarcoglycan KO mouse. As shown in Fig.15, such mice also manifest a marked loss of α sarcoglycan protein expression, a loss which was quickly reversed by two weeks of epicatechin treatment, 1 mg/kg/twice a day, potentially representing compensatory sarcoglycan expression.

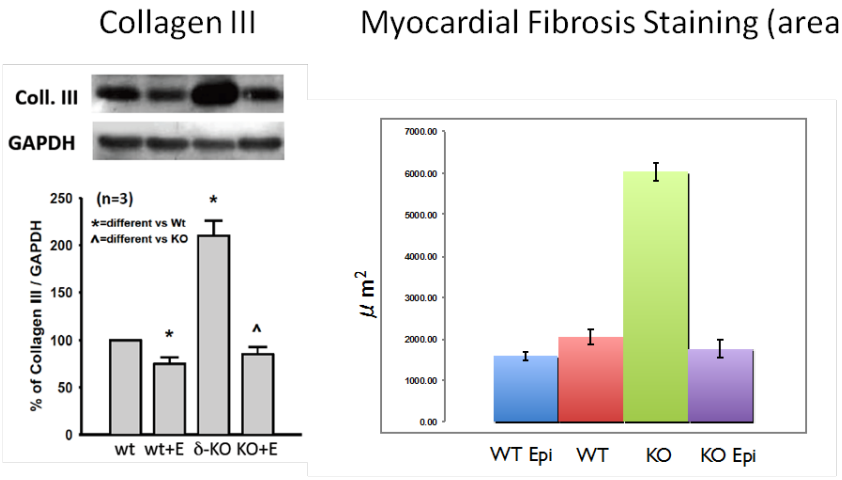
Figure 15: (-)-Epicatechin increases alpha-sarcoglycan protein expression in gamma-sarcoglycan knock out mouse muscle.



4.1.10 (-)-Epicatechin Reduces Fibrosis in δ-Sarcoglycan KO Mice (unpublished data)

Treated animals from the experiment described above also showed a dramatic decrease in expression of collagen III, a marker of fibrosis (Fig 16, left panel). The reduction in collagen III translated into reduction of myocardial fibrosis as judged by quantitative histology of heart valve tissue sections (Fig 5.13, right panel). These data suggest the potential of (-)-epicatechin to reduce cardiomyopathy often associated with MD.

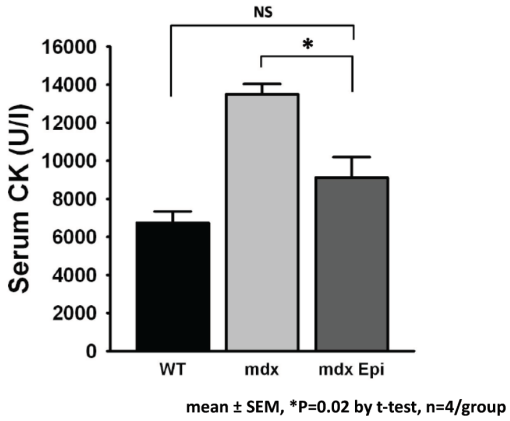
Figure 16: (-)-Epicatechin reduces fibrosis in the gamma-sarcolgycan knock out mouse.



4.1.11 (-)-Epicatechin Reduces Plasma Marker of Muscle Damage and Improves Muscle Strength in MDX Mouse Model of MD (unpublished data)

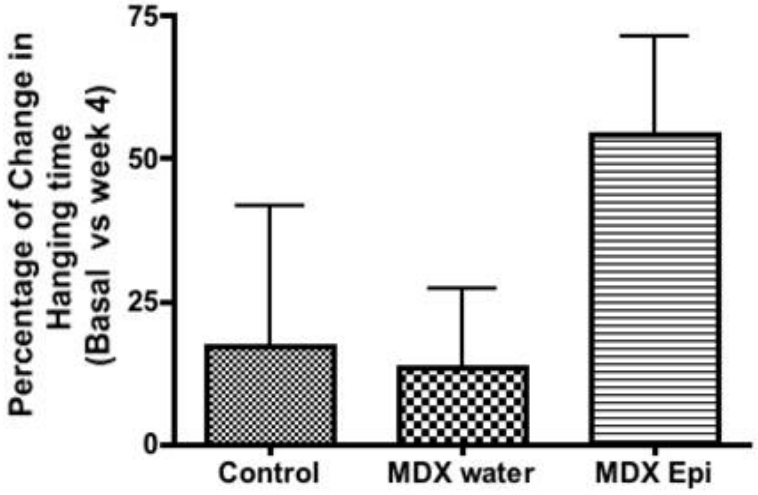
In order to confirm ongoing muscle injury in the mdx mouse model and assess the effect of (-)-epicatechin on plasma creatine kinase (CK), mice were treated with water or (-)-epicatechin (1mg/kg, bid) by oral gavage for 4 weeks; normal mice (WT) were treated with water. Excess activity of CK in plasma is recognized as a marker of skeletal and/or cardiac muscle damage. As shown in Fig 17, the levels of CK activity rise in water treated mdx mice and they are significantly reduced with epicatechin treatment to levels similar of those obtained in wild type animals.

Figure 17: (-)-Epicatechin reduces plasma CK activity (muscle injury biomarker) in *MDX* mouse model of muscular dystrophy.



In a separate study to evaluate the effect of (-)-epicatechin on muscle strength, mdx mice were treated with (-)-epicatechin (1mg/kg, qd) by oral gavage for 4 weeks; normal mice (control) were treated with water. Muscle strength testing was performed at baseline and at 4 weeks, consisting of measuring hang time of each mouse from a wire grid (30), with percent change from baseline calculated for each mouse. Figure 18 depicts (mean±SD) percent change in hang time for control and mdx mice. Control and mdx mice receiving water showed modest 18% and 14% increases, respectively, while (-)-epicatechin treatment resulted in a 55% increase in hang time. Although this was a small pilot study, the results are consistent with the ability of (-)-epicatechin to improve muscle function in animals lacking dystrophin, providing initial proof-of-concept MD efficacy data.

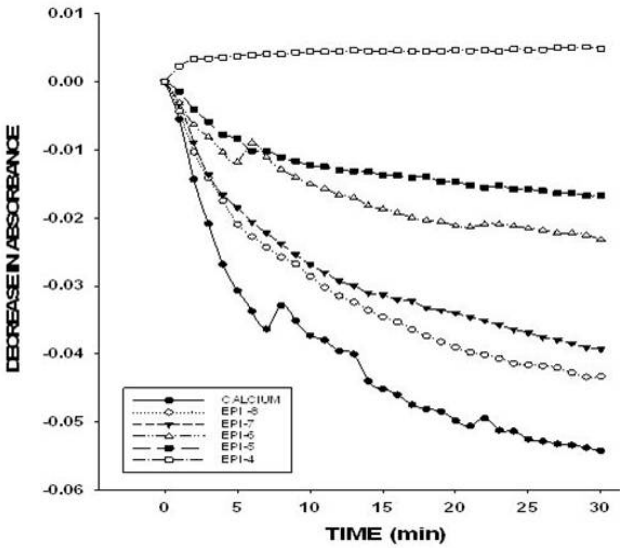
Figure 18: (-)-Epicatechin improves skeletal muscle strength in the *MDX* mouse model of muscular dystrophy.



4.1.12 (-)-Epicatechin Blocks Calcium-Induced Pore Formation in Cardiac Mitochondria (unpublished data)

Cardiac myocyte mitochondria were isolated from rat hearts and used to assess (-)-epicatechin's ability to block calcium influx and prevent damage. Mitochondrial swelling (measured by changes in light transmission) was induced by exposure to 33 μM calcium chloride alone or calcium chloride plus increasing doses of (-)-epicatechin (10^{-8}-10^{-4}M)(Figure 19). (-)-Epicatechin blocked the opening of the mitochondrial PTP in a dose-dependent manner, suggesting that (-)-epicatechin could be effective at preserving mitochondrial function in calcium-overloaded MD muscle cells and thus reducing mitochondrial-related muscle necrosis in MD patients.

Figure 19: (-)-Epicatechin blocks calcium-induced pore formation in cardiac mitochondria in a dose-dependent manner.



Epi = (-)-epicatechin. Epi-4 – Epi-8 = (-)-epicatechin concentrations of 10^{-4} – 10^{-8}M.

5.HUMAN STUDIES

5.1 Clinical Study #1: Mitochondrial Biogenesis and Muscle Improvement in Patients with Heart Failure and Type 2 Diabetes Patients (UCSD)

A proof of concept (POC) study was performed at UCSD in five heart failure, type 2 diabetes mellitus patients using (-)-epicatechin rich cocoa (mixture of a beverage and chocolate) in order to assess the effects on skeletal muscle mitochondria structure, indicators of mitochondrial biogenesis and SkM proteins and structure [58]. Published reports indicate that in patients suffering from heart failure or diabetes there is a significant compromise in mitochondrial structure and muscle bioenergetics [59-62]. Thus, altered mitochondrial bioenergetics may explain (at least in part) the impaired exercise capacity that is present in these patient populations. Changes were assessed in protein and/or activity levels of oxidative phosphorylation proteins, porin, mitofilin, nNOS, nitric oxide, cGMP, SIRT1, PGC-1α, Tfam, and mitochondria volume and cristae abundance by electron microscopy in skeletal muscle biopsies. Apparent major losses in normal mitochondria structure were observed before treatment.

5.1.1 Markers of Mitochondrial Biogenesis, Structure and Function

Fig 20 demonstrates that (-)-epicatechin-rich cocoa increased active PGC-1α with correlative decreases in acetylated (inactive) PGC1α, consistent with induction of mitochondrial biogenesis. This upregulation in mitochondrial biogenesis correlated with increased mitochondrial protein expression and cristae abundance (Figure 21).

Figure 20: (-)-Epicatechin-rich cocoa increases active PGC-1a and decreases acetylated (inactive) PGC1a in HF/DM2 patients.

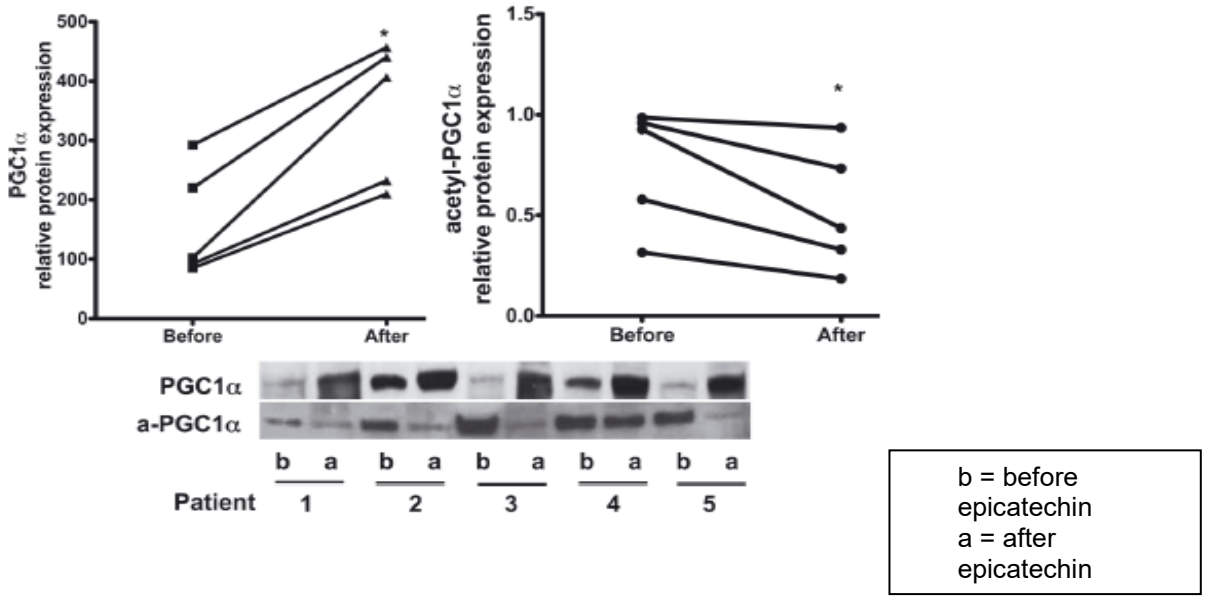
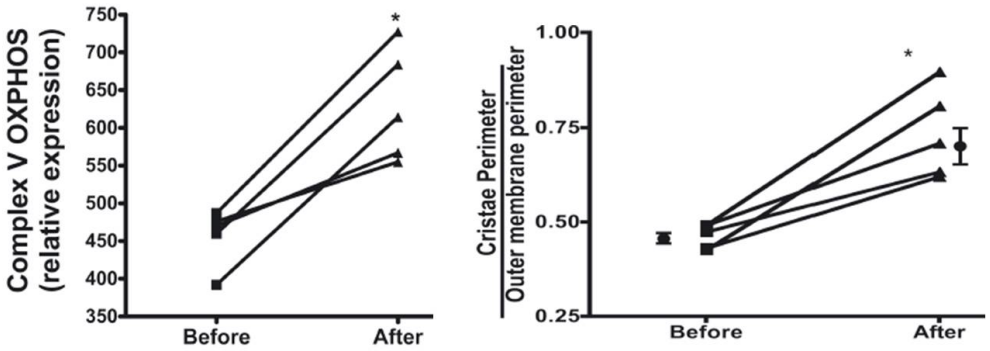
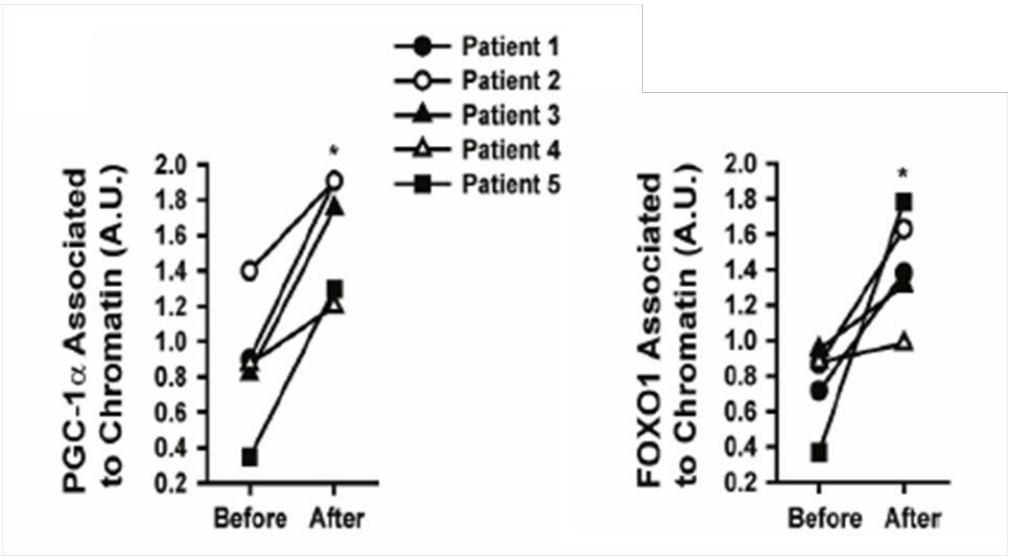


Figure 21: (-)-Epicatechin-rich cocoa increases mitochondrial complex V protein and cristae 25bundance in HF/DM2 patients.



The same patients demonstrated a marked increase in PGC-1α localized to the nuclear chromatin (22). There was a concurrent co-localization of Fox01, a cofactor with PGC-1α for inducing transcription of endogenous anti-oxidant pathways (and an important regulator of muscle energy metabolism) [63]. One of the characteristics of muscles in sarcopenia is evidence of marked oxidative injury. These anti-oxidant enzymes (e.g., superoxide dismutase, catalase, see Fig 23) would be predicted to have a beneficial effect on the injured muscle of muscular dystrophy.

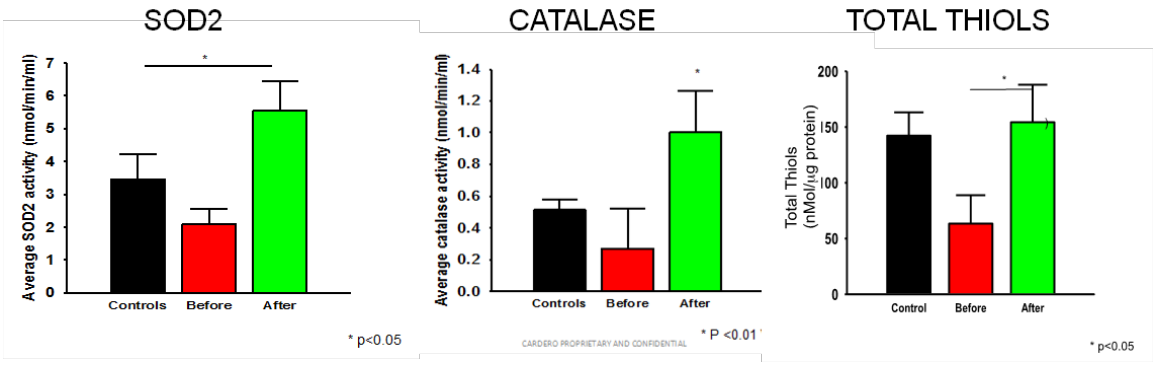
Figure 22: (-)-Epicatechin-rich cocoa increases PGC1a and FOX01 association to chromatin from quadriceps of HF/DM2 patients.



5.1.2 Markers of Tissue Oxidative Stress

Using the same group of patients but incorporating into the study a group of control subjects of similar ages (n=4) markers of tissue oxidative stress were compared before and after treatment with (-)-epicatechin-rich chocolate (Fig 23).

Figure 23: (-)-Epicatechin-rich cocoa increases antioxidant proteins and total tissue thiols (reduced glutathione) in HF/DM2 patients.

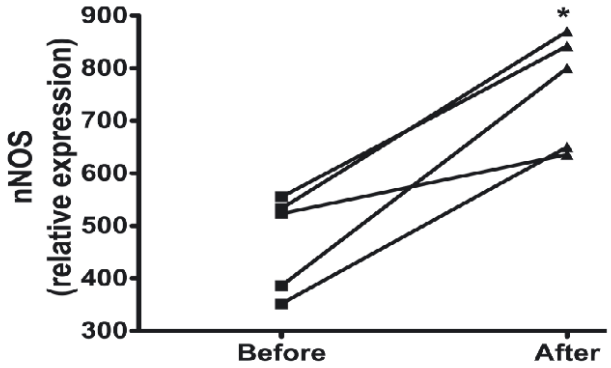


SOD2 and catalase expression were restored to levels exceeding age-matched controls and approaching levels normally associated with the young. A significant ~60% decrease in SkM tissue thiol levels were observed before treatment and these were fully restored to levels similar to controls by (-)-epicatechin-rich chocolate. Protein nitrosylation levels (determined using a slot blot) can be used as a general indicator of tissue oxidative stress levels. SkM samples of control and HF/DM2 patients were examined (data not shown). Prior to treatment, patient protein nitrosylation levels were significantly higher by ~2.5 fold than controls (=100%) and were restored to ~1.5 fold by treatment with (-)-epicatechin-rich chocolate. Altogether these results indicate that the use of (-)-epicatechin-rich chocolate has a potential to restore tissue mitochondria structure and oxidative stress levels back towards control.

5.1.3 nNOS Expression

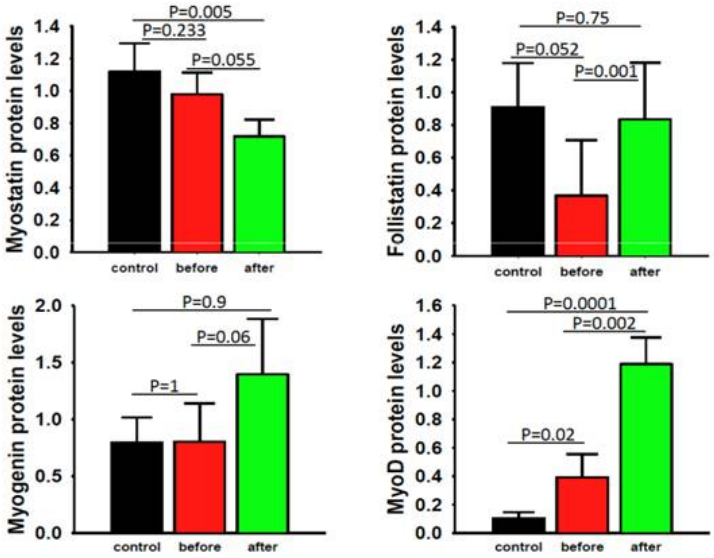
Patient muscle biopsies were assessed for nNOS expression, as myopathy, particularly BMD, is often associated with both depletion of neuronal NO synthase (nNOS) and its dislocalization from the sarcolemma. Loss of nNOS, in turn, is linked with impaired mitochondrial biogenesis, increased muscle atrophy and autophagy. Depletion and dislocalization of nNOS has a negative impact on muscle function in muscular dystrophy, causing increased muscle fatigability and functional ischemia during exercise[10, 64]. In this clinical trial, HF/DM2 patients treated with (-)-epicatechin-rich chocolate showed a statistically significant increase in quadriceps muscle nNOS expression compared to pre-treatment baseline (Fig 24).

Figure 24: (-)-Epicatechin-rich cocoa increases quadriceps muscle nNOS expression in HF/DM2 patients.



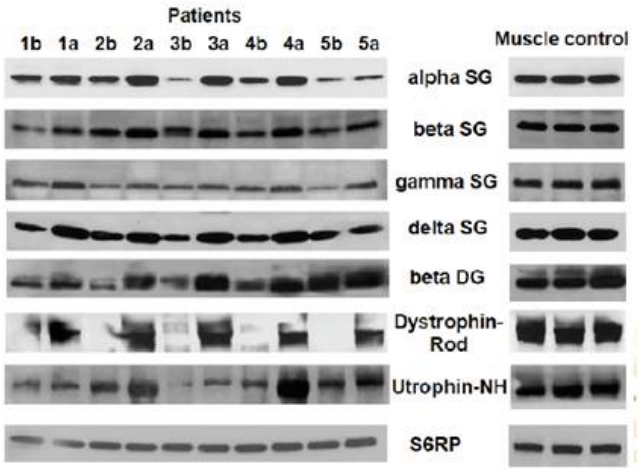
Muscle Protein Expression and Sarcomere Organization Patient SkM biopsies were evaluated for levels of muscle differentiation and structural proteins, and for sarcomere organization (unpublished data). Similar to the results of studies in C2C12 cells and mice, treatment with (-)-epicatechin increased the expression of proteins involved in muscle differentiation (follistatin, myogenin, MyoD), while levels of the inhibitor myostatin decreased (Figure 25).

Figure 25: Muscle growth/differentiation protein expression +/- (-)-epicatechin-rich cocoa in HF/DM2 patients.



Biopsies from the HF/DM2 patients were analyzed by Western blot to assess the effect (-)-epicatechin-rich cocoa on the dystrophin-associated protein complex (DAPC), a key structural component in muscle cells. As shown in Fig 26, prior to treatment there was a striking deficiency of dystrophin, which recovered to control levels with treatment. Significant increases with treatment were also noted in all the sarcoglycans, beta-dystroglycan and utrophin.

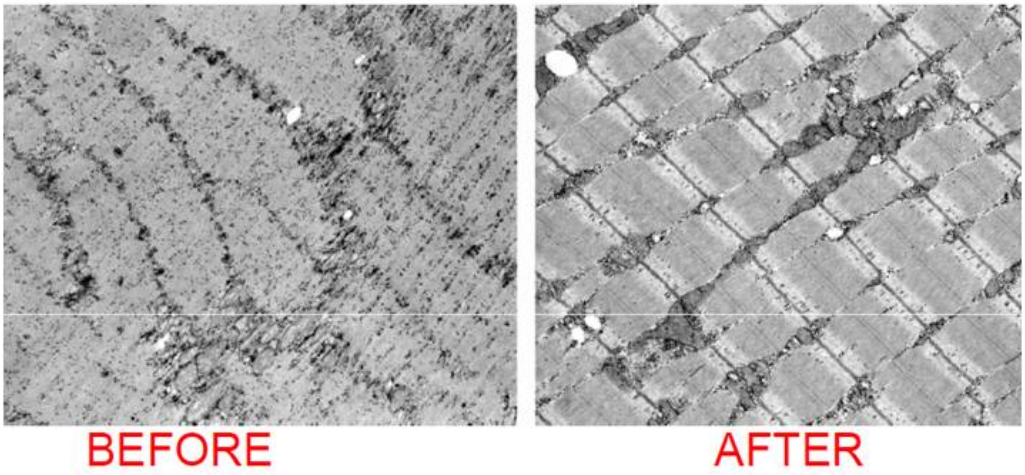
Figure 26: Muscle structural proteins induced by (-)-epicatechin-rich cocoa in HF/DM2 patients.



HF/DM2 patients before (b) and after (a) 30 day treatment with epicatechin-rich cocoa. Muscle control = healthy volunteers. SG: sarcoglycans, DG: dystroglycan

As the perturbation of dystrophin levels can greatly compromise sarcomere organization and thus, contractile function, SkM samples were evaluated using electron microscopy. Figure 27 shows a representative sample from one patient obtained before *vs* after treatment. As observed, at baseline sarcomere “organization” was very poor (no clear definition of Z and other bands). With treatment, sarcomere organization markedly recovered. Results were “quantified” using blinded, naïve graders and a highly significant improvement (P<0.0001) was observed in sarcomere organization (1.7±0.5 before vs. 3.1±0.5 after out of a maximum scale of 4) after scoring 100 EM images (10 before, 10 after/patient).

Figure 27: Changes in sarcomere ultrastructure in representative HF/DM2 patient before and after treatment with (-)-epicatechin-rich cocoa.



5.2 Clinical Study #2: Age-Related Changes in Muscle Regulatory Proteins and Effect of (-)-Epicatechin on Muscle Function and Proteins in Healthy Volunteers (UCSD)

A two part study was conducted to evaluate age-related muscle changes and the effects of (-)-epicatechin on those changes (manuscript submitted for publication, 2012). In the first part, tissue bank SkM samples were obtained from young (28.5±7 years, n=6) and old (62±2 years, n=6) subjects and analyzed for the amounts of muscle regulatory proteins. Levels of SkM growth (myostatin, follistatin), differentiation (myogenin, MyoD, MEF2A, Myf5) and senescence (senescence-associated β-galactosidase: SA-β-Gal) proteins in each group are shown in Figure 28. Muscle levels of myostatin (differentiation inhibitor) and SA-β-Gal (senescence) were increased in the older group, while those of follistatin, MyoD and myogenin decreased, suggesting an age-related loss of muscle growth and differentiation potential. These data defined a profile of age-related changes in muscle regulatory proteins and informed the selection of muscle protein outcome measures to be used in the second part of the study: (-)-epicatechin treatment of middle aged subjects.

In part 2, a small pilot treatment study was performed in human subjects (n=6, average age 41±5 years) to assess the effects of (-)-epicatechin on muscle strength and blood levels of myostatin and follistatin. Subjects were treated for 7 days with 25 mg of (-)-epicatechin in capsules BID. Muscle strength was assessed by hand grip dynamometry (thrice with each hand, alternating hands between trials and resting for 10 seconds in order to prevent fatigue, maximum strength attained was used for analysis). Myostatin and follistatin was measured in blood samples taken before and after the 7 days of treatment. Treatment resulted in a bilateral increase in hand strength of ~7% which was accompanied by a significant increase in the ratio follistatin/myostatin (Figure 29). This coupling of functional and molecular changes suggests that (-)-epicatechin has potential as a therapy to reduce progressive muscle loss.

Figure 28: Age-related muscle protein changes in human skeletal muscle samples.

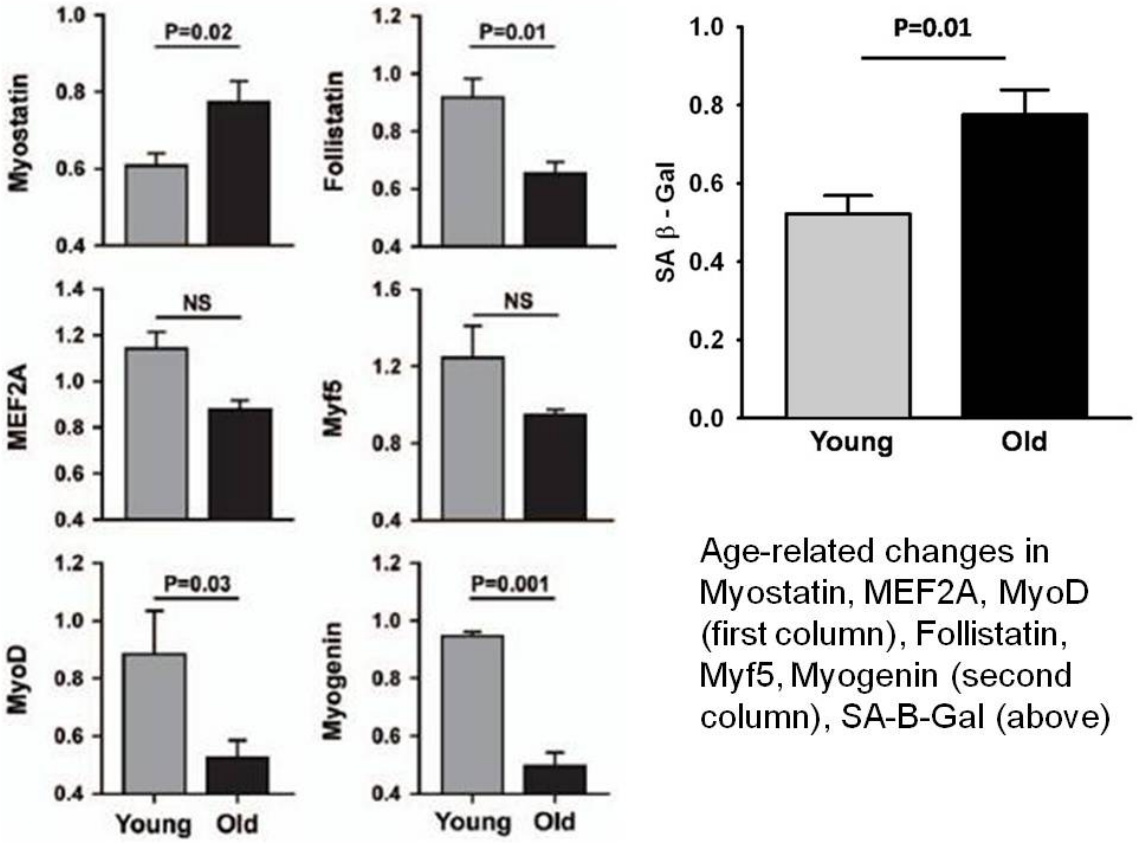
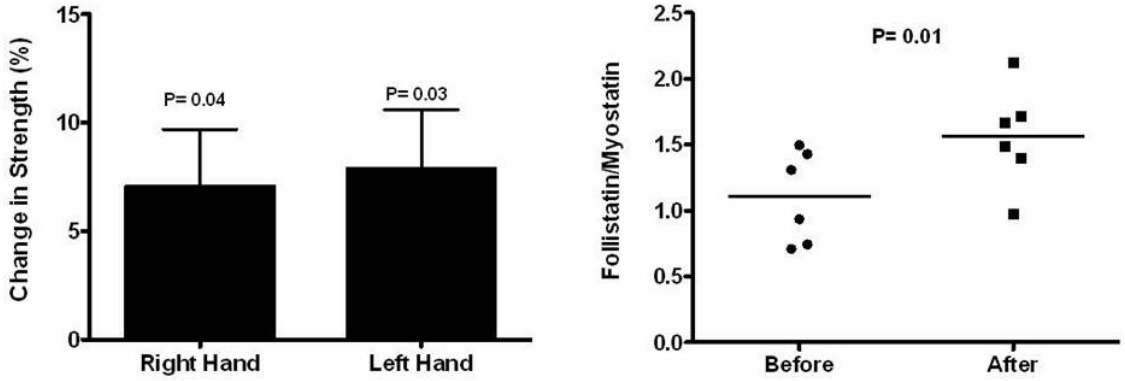


Figure 29: Changes in muscle strength and follistatin/myostatin ratio following 7 day of (-)-epicatechin treatment.



5.3 Clinical Study #3: Pharmacokinetics of (-)-Epicatechin in Healthy Volunteers (UCSF)

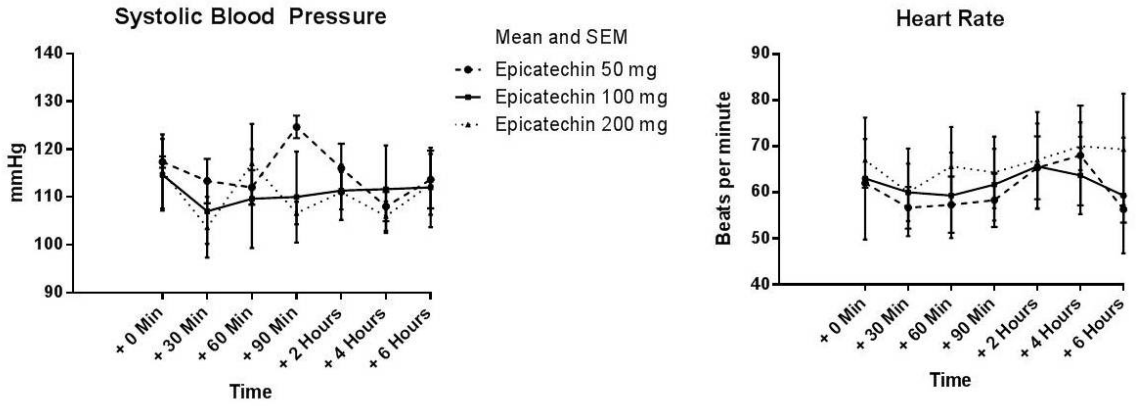
An open label study in was carried out by Dr. Christopher Barnett at the University of California, San Francisco (UCSF) to assess the safety and pharmacokinetics of a single dose of purified (-)-epicatechin in healthy volunteer subjects (n=9). (-)-Epicatechin doses of 50, 100 or 200 mg were administered in 40 ml of water.

5.3.1 Safety and Tolerability

No adverse effects were reported or observed in any of the 9 subjects. Subjects specifically denied symptoms of light headedness, dizziness, fatigue, chest discomfort or changes in breathing during the observation period. Data from vital sign monitoring is shown in Figure 30.

At time +0.5 hours there was a trend towards reduced mean systolic blood pressure with a concomitant decrease in heart rate that is consistent with subjects being relaxed, and not with a blood pressure lowering from (-)-epicatechin. In one subject blood pressure at time +0.5 hours decreased markedly, but the value was obtained immediately upon waking, the subject denied symptoms and the heart rate was also reduced from baseline, findings that are consistent with relaxation and not (-)-epicatechin induced vasodilatation.

Figure 30: Vital signs following (-)-epicatechin administration in healthy subjects.



5.3.2 Pharmacokinetics

Pharmacokinetic parameters for each dose administered are summarized presented in detail in the investigator's brochure. The (-)-epicatechin concentration and metabolite concentration rose rapidly following oral administration consistent with rapid absorption and first pass metabolism as has been previously described [34, 37-44, 46]. Complete plasma concentration profiles of (-)-epicatechin and its metabolites after oral administration can be found in the complete PK study report in the investigator's brochure. The shape of the natural log transformed plasma concentration curves was consistent with first order pharmacokinetics.

The ratio of the AUC for (-)-epicatechin and each metabolite to administered dose of (-)- epicatechin is given in Table 6.4. The AUC/dose ratio remains nearly constant for (-)-epicatechin but increases with increasing dose of each of the metabolites suggesting either dose dependent excretion of these metabolites, or perhaps saturation of some competing pathway for epicatechin metabolism. Investigator brochure Table 6.5 shows that $AUC_{metabolite}/AUC_{epicatechin}$ is high for each of the metabolites suggesting that one or more of the metabolites may be an important mediator of biological effects.

5.3.3 Conclusions

(-)-Epicatechin was well tolerated over the 50-200 mg dose range, with rapid absorption and first pass metabolism. Non-metabolized (-)-epicatechin represented <5% of the total (-)-epicatechin compounds by 30 minutes. (-)-Epicatechin and its metabolites were rapidly cleared from the body with a plasma elimination half-life of approximately 2.5 hrs for the 100 and 200 mg doses. Plasma concentration of (-)-epicatechin was generally proportional to the administered dose. The 3 dominant groups of metabolites in our study were glucuronidated, sulfated and methyl sulfated, consistent with a recently published evaluation of (-)-epicatechin pharmacokinetics and metabolism [37]. A novel finding in this study was that the $AUC_{Metabolite}/AUC_{epicatechin}$ ratio increased with increasing doses suggesting dose dependency of (-)-epicatechin metabolism.

5.4 Clinical Study #4: Epicatechin in Becker phenotype dystrophinopathy patients.

As a proof of concept in adults with dystrophinopathies, we conducted a single-center open-label proof-of-concept pilot study of oral epicatechin 50mg twice daily in ambulatory adults with genetically-confirmed Becker muscular dystrophy from August 2013 to June 2014.

Study participants: This was a single-center proof-of-concept pilot study and due to the limited availability of both genetically-confirmed BMD patients and a limited drug supply, we employed a convenience sample for this study. The main criterion for success of the study was presence of one or more biologic or strength and performance outcome measures that yield a response magnitude that allows for sufficient power in a Phase II B study with a sample size of 30 individuals. A maximum of ten participants were to be enrolled in our study. Participants were males between the ages of 18 and 60 years with a genetically-confirmed diagnosis of BMD, average to low daily physical activity and the ability to ambulate at least 75 meters without the use of assistive devices.

Study drug: Participants received epicatechin by mouth 50mg twice per day (100mg per day total dose) for 8 weeks.

Study procedures and evaluation: Participants had a total of 7 study visits each at baseline and at screening, day 1, and weeks 1, 2, 4 and 8. A comprehensive safety review was conducted at each visit, and a medical monitor reviewed suspected study-related adverse events throughout the duration of the study. Evaluations of efficacy are shown in the table below.

Table 2: BMD epicatechin pilot study efficacy evaluations.
1) assessment of peripheral blood biomarker profiles;
2) assessment of baseline and post-treatment muscle biopsy by histology, Western blot and electron microscopy, and;
3) assessment of strength by isokinetic dynamometry and quantitative grip testing [7, 65], 4) exercise performance and metabolic testing during a standardized graded recumbent cycle test[66, 67],
5) muscle perfusion of the vastus lateralis by NIRS during the exercise test [68], and
6) the 6-minute walk test.

5.4.1 Results

Table 3: Participant characteristics at baseline.

Measure	N	Mean	SD	Min	Max
Age at screening (Yrs)	7	46.6	9.5	31.5	60
Height (cm)	7	179.2	4.3	172.5	185.5
Weight (kg)	7	83.2	18.3	53.3	104.5
Forced Vital Capacity (%)	7	93.4	12.5	77	116
6MWD (m)	7	372	87.2	245	502
Time to Stand (s)	5	5.5	2.3	2.9	8.1
Time to Climb 4 Stairs (s)	7	7.4	7.4	4.4	12.3
Time to Run 10m (s)	7	7.9	7.9	5.1	12.8

Recruitment and baseline data: Seven participants with sufficient genetic diagnostics passed screening. One participant who failed screening was not sufficiently ambulatory to perform the required functional evaluations. One participant withdrew consent during screening. The remaining participants all began treatment. Baseline characteristics of the final study cohort are shown in **Table 3**. One participant was unable to participate in the full set of strength testing measures at baseline due to the advanced level of his disease. Tissue for electron microscopy was limited to 3 patients due to poor tissue quality and an error in tissue fixation for one sample.

5.4.2 Biomarker outcomes at 8 weeks

All enrolled participants completed 8 weeks of follow-up on study medication. One participant sustained a fall at home (unrelated to study medications) that resulted in a minor knee injury and temporary limitation of mobility just prior to his 8-week evaluation and was unable to undergo week 8 exercise testing or biopsy.

Biomarkers of mitochondrial biogenesis from muscle biopsy tissue: Notable changes in muscle tissue as measured by Western blot included significant increases in transcription factors LKB1, PGC1a, AMPK, and mitofilin associated with mitochondrial biogenesis and function **(Figure 1).** We were able to collect bicep muscle tissue of sufficient quality to directly image mitochondria for 3 participants. Those participants showed increases in both numbers of mitochondria and cristae abundance supportive of our overall concept **(Figure 2)**. One participant demonstrated approximately double the number of muscle mitochondria per unit area after 8 weeks of treatment **(Figure 2, first panel)**.

Figure 1: Increased muscle / mitochondrial transcription factors.

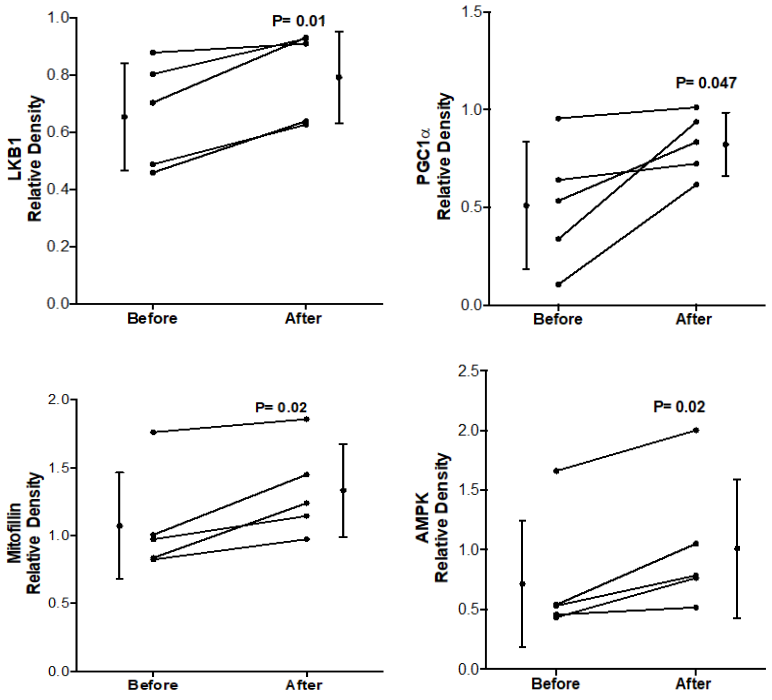
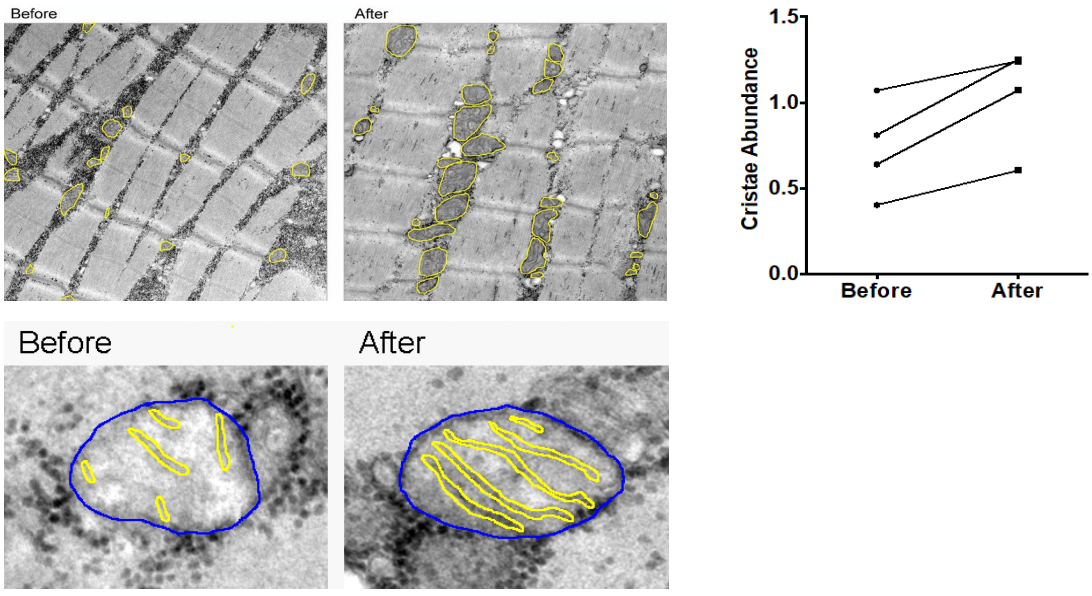
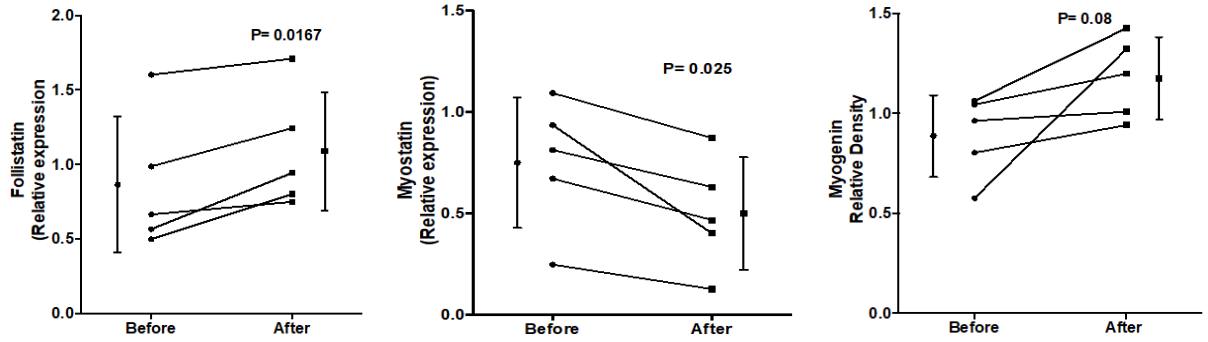


Figure 2: Increases in mitochondrial count and cristae abundance in 4 participants with viable muscle biopsies.



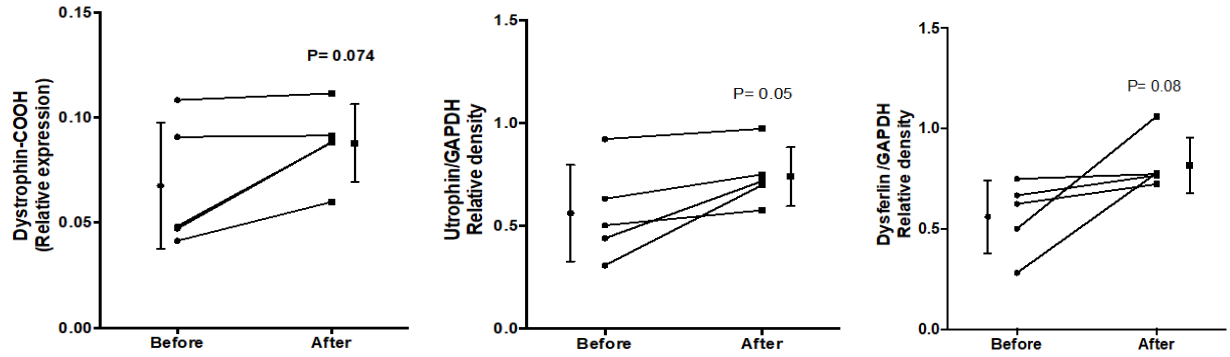
Biomarkers of regeneration from muscle biopsy tissue: Western blot results from muscle biopsy also indicated significant alterations in expression of tissue follistatin and myostatin **(Figure 3)**, which are major regulators of muscle growth and repair, suggesting a shift toward proliferation of mitochondria and muscle tissue overall.

Figure 3: Pro-repair regulation of muscle growth and regeneration-related proteins.



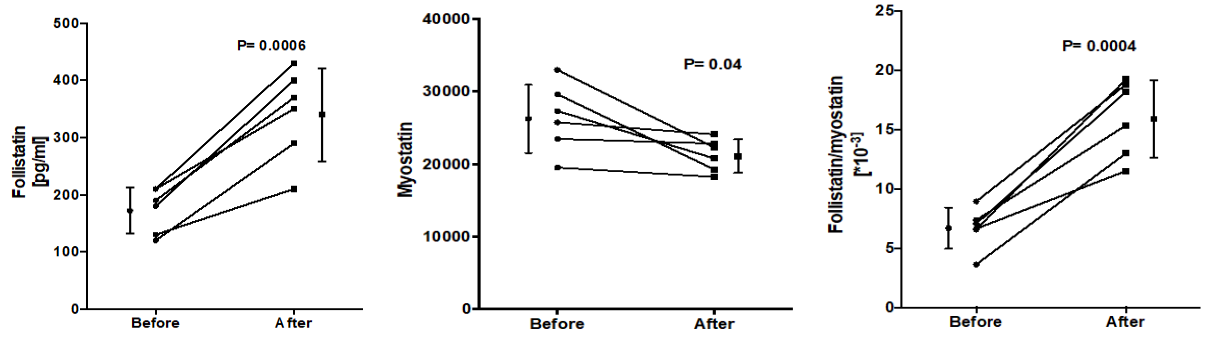
Dystrophin-related proteins from muscle biopsy tissue: Increases were seen in muscle tissue Western blot for key structural proteins dystrophin, utrophin and dysferlin (**Figure 4**), with non-significant increasing trends in other related skeletal muscle proteins suggesting a downstream pattern of growth and repair that is consistent with the previously mentioned increases in regulatory proteins.

Figure 4: Increased dystrophin-associated proteins in muscle by Western blot.



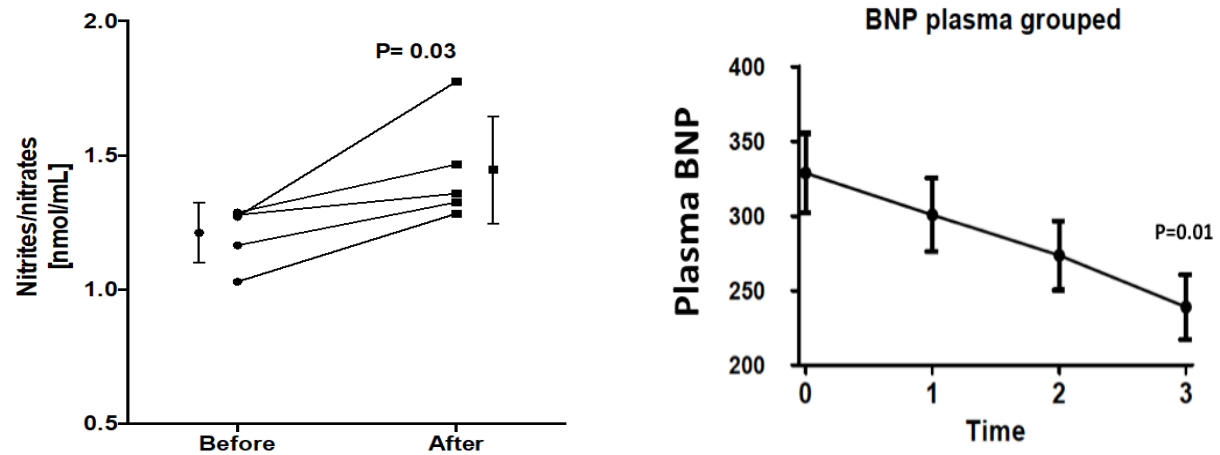
Biomarkers of muscle regeneration and mitochondrial growth from plasma: Changes in muscle tissue follistatin and myostatin were replicated in circulating plasma as well, with increased follistatin and decreased myostatin expression. The striking change in the ratio between plasma levels of growth potentiating follistatin and growth inhibiting myostatin suggests a likely biomarker of initiation of a tissue repair state **(Figure 5)** that will be useful in future clinical trials. Mass spectrometry yielded statistically significant increases ($p<0.05$) of between 8-fold and 140-fold in mitochondrial proteins acyl-coenzyme A thioesterase 2, acyl-CoA synthetase family 3, mitochondrial chaperone BCS1, paraplegin, 28-s ribosomal protein S31 (mitochondrial) and threonine synthase-like 1, all of which are indicators of upregulated mitochondrial production.

Figure 5: Follistatin and myostatin changes in circulating plasma act as a biomarker of muscle growth and repair.



Biomarkers of heart failure and NO reserve from plasma: In addition to indicators of improved energetics and muscle repair, we demonstrate significant increases in plasma nitrates, including nitric oxide (NO), a key regulator of vascular tone that promotes tissue oxygenation during exercise **(Figure 6)**. Nitric oxide synthetase (NOS), which is responsible for NO production, co-localizes with dystrophin and is known to be reduced in individuals with dystrophinopathies. We also noted significant reductions in plasma B-natriuretic peptide (BNP) which is expressed during heart failure and by skeletal muscle satellite cells in response to ischemic stress **(Figure 6)** [69]. These results again suggest an overall pattern of growth and repair and normalization of function.

Figure 6: Changes in NO and BNP expression in plasma show reduced ischemic stress.

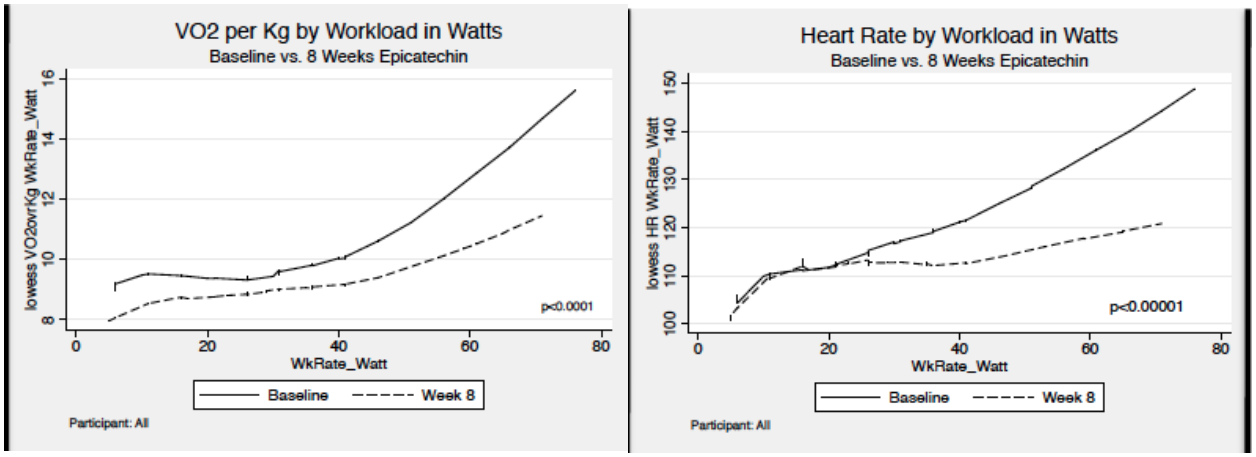


5.4.3 Strength and function and safety outcomes at 8 weeks.

Metabolic indicators during graded exercise cycle testing: Overall, duration of exercise and maximal attainable workload did not increase on the graded cycle exercise test. We did observe significant improvements in metabolic exercise performance that are consistent with and similar in magnitude to those seen in response to short-duration (8-10 weeks) exercise training regimens. During the graded cycle exercise test, participants demonstrated significant decreases from baseline at 8 weeks in VO2, blood lactate and heart rate for a given level of workload in watts (**Figure 7**) indicating a reduced energy cost of exercise, and improved muscle energy utilization and respiratory efficiency. These effects are highly consistent with our biological marker, and further support our concept that supplementation with epicatechin provides an exercise training-like effect for muscle in people with dystrophinopathies in a manner consistent with moderate exercise but without the damaging mechanical stress. Participants demonstrated significant changes in tissue oxygen saturation index (TSI) by near infrared spectroscopy (NIRS) in the vastus lateralis during the six-minute walk test ($p<0.001$). These changes suggest an improved degree of vasodilation

during exercise that is consistent with the overall increase in expression of plasma NO observed in the biomarker studies.

Figure 7: Metabolic exercise test parameters show efficiency gains during exercise.



Clinical measures of strength and function: Despite metabolic testing evidence demonstrating reduced work of exercise via V02, lactate and heart rate reductions at a given level of work, we failed to show significant groupwise improvements in commonly-used clinical measures of timed motor performance, strength and pulmonary function that we included in our evaluation set. However, our participant who demonstrated a doubling of mitochondrial volume as noted above also increased his 6MWT distance from 350m to 494m while other participants showed mild increases or stability over 8 weeks. We feel that the lack of response in clinical measures is due mostly to the short duration of the study and the small sample size. It has been demonstrated in randomized clinical drug trials in patients with more rapidly progressing Duchenne muscular dystrophy diagnoses that evidence of groupwise differences between treated and non-treated participants do not become evident until between 24 and 48 weeks of treatment [70]. Given the current evidence there is a clear indication of the need for long-term studies of epicatechins in dystrophinopathy patients.

5.4.4 Safety outcomes at 8 weeks

No serious adverse events occurred during the treatment phase of the study. Mild to moderate health events were consistent with community living (e.g. cold, headache) and were not present in a pattern that suggested that they were related to epicatechin therapy. One participant experienced a biopsy wound infection that resolved with oral antibiotics. Moderate short-duration bruising from muscle biopsies was present in all participants and was consistent with standard clinical care, and resolved without incident.

6 RESEARCH DESIGN AND METHODS

6.1 Selection and Withdrawal of Participants

6.1.1 Criteria for Enrollment

Up to ten participants will be studied in this initial pilot project.

Participant Inclusion Criteria

- Prior participation in UCD0113 BMD epicatechin pilot study
- Male
- Age 18 years to 70 years
- Average to low daily physical activity
- Ability to ambulate for 75 meters without assistive devices
- Diagnosis of BMD confirmed by at least one the following:

  - Dystrophin immunofluorescence and/or immunoblot showing partial dystrophin deficiency, and clinical picture consistent with typical BMD, or
  - Gene deletions test positive (missing one or more exons) of the dystrophin gene, where reading frame can be predicted as 'in-frame', and clinical picture consistent with typical BMD, or
  - Complete dystrophin gene sequencing showing an alteration (point mutation, duplication, or other mutation resulting in a stop codon mutation) that can be definitely associated with BMD, with a typical clinical picture of BMD, or
  - Positive family history of BMD confirmed by one of the criteria listed above in a sibling or maternal uncle, and clinical picture typical of BMD.
- Nutritional, herbal and antioxidant supplements taken with the intent of maintaining or improving skeletal muscle strength or functional mobility have been discontinued at least 2 weeks prior to screening (daily multivitamin use is acceptable).
- Hematology profile within normal range
- Baseline laboratory safety chemistry profile within normal range
- No plan to change exercise regimen during study participation

Participant Exclusion Criteria

- Currently enrolled in another treatment clinical trial.
- History of significant concomitant illness or significant impairment of renal or hepatic function.
- Use of regular daily aspirin or other medication with antiplatelet effects within 3 weeks of first dose of study medication.
- Regular participation in vigorous exercise.
- Symptomatic heart failure with cardiac ejection fraction <25%

6.1.2 Participant Screening Schedule

The entry procedures will be conducted according to the schedule in **Table 3**. After potential participants have completed the screening visits and the site has verified they meet all study inclusion criteria the participant will be entered into the treatment phase of the study.

Table 3: Study procedures

Table __ : (Study Matrix) Open-label extension study of epicatechin in adult Becker muscular dystophy.

Assessments		Stage 1: Dose Finding Phase			Stage 2: Medium Term Outcomes		Stage 3: Long-Term Outcomes		
		Screening	Baseline	PK Week 4	Week 8	Week 12	Week 24	Week 36	Week 48
		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
		Day -28 to 0	Day 1	Day 28	Days 53-59	Day 81-87	Day 165-171	Day 249-255	Day 333-339
Medication Dispensing / Administration			X	X	X	X	X	X	X
Safety	Medical History	X	X	X	X	X	X	X	X
	Physical/Neurological Examination	X	X	X	X	X	X	X	X
	AE/Concomitant Medications	X	X	X	X	X	X	X	X
	Electrocardiogram	X	X	X	X	X	X	X	X
	Laboratory Safety								
	Complete Blood Count	X		X	X	X	X	X	X
	Serum Chemistries	X		X	X	X	X	X	X
	Urinalysis	X		X	X	X	X	X	X
	Fasting Insulin, Glucose	X				X	X	X	X
Pharmacokinetics			Pre, 2H, 4H	Trough		Trough	Trough		Trough
Efficacy									
	Biomarkers								
	Plasma Follistatin		X	X		X	X	X	X
	Plasma Myostatin		X	X		X	X	X	X
	Plasma Follistatin:Myostatin Ratio		X	X		X	X	X	X
	Plasma Nitrites / SNO		X	X		X	X	X	X
	Plasma BNP		X	X		X	X	X	X
	Plasma Creatine Kinase		X	X		X	X	X	X
	Plasma MMP-9		X	X		X	X	X	X
	Plasma TNF-Alpha		X	X		X	X	X	X
	Plasma TGF-Beta		X	X		X	X	X	X
	Proteomics (Exploratory)								
	Serum Mass Spectrometry (exploratory)		X	X		X	X	X	X
	Somalogic biomarker assay		X				X		X
	Clinical Exercise Testing								
	6-minute walk test w/metabolic testing / TSI		X	X		X	X	X	X
	Graded cycle exercise test w/metabolic testing / TSI		X	X		X	X	X	X
	Timed motor performance (standing, stair climb, 10-meter run/walk)		X	X		X	X	X	X
	Qantitative Strength Testing (Knee/Elbow Flexors/Extensors, hand grip)		X	X		X	X	X	X
	DEXA Body Comp		X	X		X	X	X	X
	Person-Reported Outcomes								
	POSNA PODCI		X	X		X	X	X	X
	PROMIS/NeuroQoL		X	X		X	X	X	X

6.1.3 Randomization

This is an open-label pilot study, and will not utilize randomization in the study design.

6.2 Withdrawal of Participants

Participants are free to withdraw from the study at any time. Study investigators may elect to withdraw a participant from the study for reasons including an adverse event and inability to comply with study procedures. Reasons for withdrawal of all participants will be recorded for review by the Study Medical Monitor.

6.2.1 Follow-Up of Withdrawn Participants

Those participants who are withdrawn from study medication, but are still willing to finish study participation per protocol, will be followed with all specified testing for the remainder of the 48-week study period. If participant agrees to follow-up, adverse event and medical event follow-up, along with basic safety data, will also be collected at scheduled study visits or via phone every *2 weeks* for the remainder of the study period (through week 48).

6.3 Treatment of Participants

6.3.1 Epicatechin administration

Participants will receive epicatechin by mouth 50mg twice per day (100mg per day total dose). Study medication will be supplied as a clear 25mg (#3) gelatin capsule without any inert fillers. Participants will

take two capsules in the morning at approximately 7:30AM at least 15 minutes before the morning meal and two with the evening at approximately 7:30PM at least 1 hour after the evening meal as absorption is limited by some milk products.

For details on participant enrollment, please refer to the study procedures chart (Table 3) and pharmacy sections. When medications have been received, they should be dispensed to participants from their assigned investigational drug supply along with the medication instructions handout for parents and physicians.

6.3.2 Criteria for Dose Reductions

Dose reductions will occur due to adverse drug events and participants will not have their doses re-escalated during the course of the study. Criteria for dose reduction will include:

- Recurrent non-manageable headache
- Increase in baseline safety evaluations >1.5 ULN or 100% change from baseline, excepting CK, AST, ALT
- Abnormal coagulation studies (PT/PTT)
- Other Grade 4 adverse event

If participants require a dose reduction based on the criteria listed above follow the guidelines in Table 4 when reducing a participant's study medication dose.

Table 4: Dose reduction schedule.

Dose Reduction 1	Reduce participant's dose by 50mg/day (25mg AM, 25mg PM dose).
Dose Reduction 2	Withdraw participant from study medication

The Common Terminology Criteria for Adverse Events (CTCAE) published by the Cancer Therapy Evaluation Program will be used to grade adverse events for this trial.

6.3.3 Study Drug Holiday as Result of Surgical Procedure

If a participant experiences an SAE that necessitates an unanticipated surgical procedure, the study drug should be withdrawn and a "drug holiday" begins. The day the study drug is withdrawn (first day not taking study drug) is Day One of the drug holiday. A drug holiday can last up to 2 weeks (14 calendar days).

Study drug should be withdrawn 2-5 days prior to the scheduled surgery date. The study drug should be restarted 2 days post surgery unless PI has medical rational for other regimen for restarting study drug.

6.3.4 Concomitant Therapy

No new medications may be taken, except over the counter cold remedies, daily multivitamin, and Zantac, during the study period without the agreement of the Study Chair. The exception is any case where such medications are used to prevent injury or disability due to unforeseen adverse events.

6.3.5 Patient Care Outside UC Davis

Participants participating in UC Davis studies may not receive protocol therapy at non-UC Davis institutions. However, the following are acceptable:

- For interim blood work done at a non-UC Davis site, a copy of the laboratory slip or other documentation MUST be forwarded to UC Davis for inclusion in the medical record and case report forms.

These guidelines do NOT override any federal, international or sponsoring agency requirements.

Records from any non-UC Davis institutions must be available for audit.

7 PHARMACY

7.1 Dosing Information and Safety Studies

The safety of this supplement has not been proven in the BMD population. We propose to use a dose at approximately 1.1-1.4 mg/kg/day spit between the morning and evening doses. The maximum dose in our population will not exceed 100 mg/day.

7.1.1 Preclinical Rodent Safety Studies

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 [71]. 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)[72]. The intraperitoneal (-)-epicatechin median lethal dose (LD_{50}) 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 LD_{50} of above 1000 mg/kg may be regarded as safe [73].

7.1.2 Human Safety Studies

Clinical studies with purified (-)-epicatechin (1-2 mg/kg) in the dose range of Cardero's proposed studies did not reported any adverse events[42, 48]. 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 (reviewed in [74, 75]). 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 [76].

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).

7.2 Study Drug Formulation and Procurement

Epicatechin for this study will be obtained from Cardero Therapeutics, Inc. and will be supplied as 25mg clinical trial grade (#3) clear gelcaps in bulk containers. Distribution of medication will be supervised by the UC Davis Investigational Drug Service Pharmacy.

7.2.1 (-)-Epicatechin as a Natural Test Substance (Cardero Therapeutics)

(-)-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 (see Appendix) and a %-content by weight is calculated.

Chemical name:	(-)-Epicatechin or (2R, 3R)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol
Molecular formula:	$C_{15}H_{14}O_6$
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. The structure of (-)-epicatechin is depicted in Figure 1. The absolute stereochemistry of (-)-epicatechin is as shown because it is either isolated from natural sources and further purified, or it is synthesized and compared to the natural isomer.

Stability (-)-Epicatechin has been demonstrated to be stable in the lyophilized state for at least six months under refrigeration. A stability study is ongoing and retest dates will be generated based on results.

7.2.2 (-)-Epicatechin as supplies as a natural test product

The intended route of administration is oral. (-)-Epicatechin isolated from tea will be supplied by Cardero Therapeutics in gelatin capsules, each containing 25 mg (-)-epicatechin combined with excipients. The test product is used as is, and swallowed with some water. Table 5 describes the (-)-epicatechin capsule formulation.

Table 5: Contents of the 25mg (-)-epicatechin capsule.

Component	Role	Amount
Epicatechin	Natural Ingredient	25.00mg
Microcrystalline cellulose@ (Avicel PH 102)	Diluent	127.00mg
Crospovidone@ (Kollidon CL)	Disintegrant	4.80mg
Citric acid monohydrate@	Acidifying agent	1.60mg
Colloidal Silicon dioxide@	Glidant	0.80mg
Magnesium Stearate@	Lubricant	0.80mg
Total		**160.00mg**

Gelatin Capsule The gelatin has been formulated into hard gelatin capsule shells for use in human pharmaceuticals. The gelatin itself is limed bone gelatin, and has been obtained from Rousselot SAS (production site: Chemin Moulins Premiers, France – 84800 Isle-Sur-La-Sorgue). The applicant has obtained Certificate of Suitability No. R1-CEP 2000-029-Rev 03, dated 22 July 2011, from Rousselot. According to this certificate, the substance GELATIN meets the criteria described in the current version of the monograph *Products with risk of transmitting agents of animal spongiform encephalopathies* (no. 1483 of the European Pharmacopoiea).

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 (capsules) will be USP grade (vitamin C, EDTA, cellulose, lactose). Sterile water will be used to prepare liquid doses for immediate and single use.

Stability A stability study of the test product in capsules is ongoing. Retained samples of clinical trial supplies will be tested to confirm potency.

7.3 Treatment Cycles Drug Dispensation

Medications will be dispensed for the entire period of time between study visits, and will be clearly marked as investigational drugs per UC Davis Investigational Drug Service Pharmacy procedures. The site will dispense study medication/placebo to the participant and will keep a medication log to document all study medication dispensed to participants. The participant will be instructed to take the study medication per protocol dosing schedule, every day during the eight-week treatment period.

7.4 Investigational Product Accounting Procedures

At each study visit, participants will be asked to return used medication containers from the previous month(s), as well as any unused medications. The participating centers will measure any unused medication and the site coordinator will keep records of the participant's medication use.

7.5 Maintenance of Randomization Codes and Emergency Unblinding

This study is open-label and no emergency unblinding procedures are necessary.

8 ASSESSMENT OF EFFICACY

8.1 Laboratory Efficacy Parameters

- Peripheral Blood Tests: Peripheral venous blood will be collected at points specified in the study visit chart to evaluate blood biomarkers as noted in the specific aims.

8.2 Functional Efficacy Parameters

- **Anthropometric Measurements (5 minutes)**

  Standing height will be measured to the nearest 0.1cm in all participants. Weight will be measured to the nearest 0.1kg. Waist and hip circumference will be measured to the nearest 0.1cm and waist-hip ratio will be calculated.

- **Body Composition Assessment by Dual energy X-ray absorptiometry (DEXA) (15 minutes)**

  DEXA scans are primarily used to evaluate bone mineral density. It can also be used to measure total body composition and fat content with a high degree of accuracy. For this study it will be used to measure total and regional body composition. DEXA uses X-rays to assess bone mineral density or measure total body composition. However, the radiation dose is approximately $1/10^{th}$ that of a standard chest X-ray. Bone density will be evaluated based on whole-body and subcranial total bone mass and areal bone mass (adjusted mass for bone size).

- **Functional Evaluation (1 minute)**

  The functional classification used by CINRG utilizes a scale modified from the upper extremity scale reported by Brooke et al [77] and the lower extremity scales used by Vignos et al [78]. The functional grades consist of six levels of function for the upper extremities and eleven levels for the lower extremities. Specific methods are outlined in the CQMS User's Manual.

- **Upper Extremity Range of Motion Testing (60 minutes)**
  The Kinect camera is a product for Xbox designed by Microsoft that captures motion (http://msdn.microsoft.com/en-us/library/jj131033.aspx). The Kinect camera will be used to measure reachable workspace and fatigue of the participant using the protocol developed and published by Kurillo, Han et al. [79-84].
  While being monitored by the Kinect, the participant will:
  - perform a series of standardized range of motion and functional upper extremity tasks
  - perform a series of standardized range of motion and functional upper extremity tasks while holding a weight

- **A6MCT for Arms and Legs (15 minutes)**
  Participants will perform an assisted 6-minute cycling test to assess endurance as described by Jansen et all [85]. The exercise test will begin by positioning the participant on a motorized assisted mobility trainer (KPT Cycla, Kinetec, France) that can be used with a participant's personal (electric) wheelchair or a regular chair with a backrest. The goal of the test is to cycle as fast as possible and keep it up for 6-minutes. The KPT Cycla is set in passive mode 1 with no-load speed of 7 RPM. Participant are seated either in their wheelchair or a chair with back support in front of the KPT Cycla, with the hips and knees are positioned at 90 degrees of flexion for one leg while the other will be submaximally extended. The arm pedal axis of the KPT cycla will be adjusted a few centimeters (max of 5 cm) below shoulder level. The distance from the chair to the bicycle is determined by allowing participants to move their legs and arms over submaximal range of motion, producing a feeling of stretch. Verbal encouragement is given every 15 seconds. Participants are

also told of the time completed and time left every minute. Rest is allowed if fatigued but participants are encouraged to continue cycling as soon as possible. The outcome of the test is the number of revolutions achieved in 6-minutes. Revolutions per minute (cumulative) and rest periods are also recorded.

- **Graded exercise test using a recumbent cycle ergometer (45 minutes)**

  Each participant will perform a graded exercise test on an electronically braked recumbent cycle ergometer as previously described [66, 67]. Modifications for Becker muscular dystrophy related proximal weakness will be made as described by others [67, 85]. The exercise test will began by participants pedaling at a rate of 60 revolutions/min with no load for 1 min (warm up). After the warm-up period, the work rate (WR) on the cycle ergometer will start at 10 W and increase either 10 W/min or every other minute for Becker muscular dystrophy participants until volitional exhaustion. Level of exertion will be monitored by heart rate and the Borg visual analogue scale [86]. Increments will be adjusted so that the duration of the test will be kept between 12 and 15 min. Twelve-lead ECG, blood pressure, O2 consumption ($V\dot{}O2$), CO2 production ($V\dot{}CO2$), and ventilation (Vmax, SensorMedics/VIASYS Healthcare) will be continuously measured. After volitional exhaustion, participants will be immediately placed in the supine position, where a postexercise ECG will be performed within 15–30 s. Exercise tests will be considered to be maximal if the peak heart rate (HR) is 85% of that predicted for age (220 – age) and/or the peak respiratory exchange ratio (RER; $V\dot{}CO2/V\dot{}O2$) is ≥ 1.15. Muscle perfusion testing of a quadriceps muscle will be performed during cycle exercise using a non-invasive portable near-infrared spectroscopy (NIRS) system as described by Allart et al. [68]. We will use a non-invasive three channel, portable continuous-wave NIRS device (PORTAMON, Artinis Medical Systems, Zetten, The Netherlands). Participants will undergo blood lactate testing at baseline and at 2-minute intervals as specified in the “Peripheral Blood Tests” section.

- **Six-Minute Walk Test (10 minutes)**

  Participants will complete the Duchenne muscular dystrophy six-minute walk test (6MWT) as described by McDonald et al [87]. Methods will be adapted for the adult population by removing the element of constant verbal encouragement. Measurements recorded will include 25-meter split times and total distance traveled.

- **Quantitative Muscle Testing (20 minutes)**

  Isometric elbow flexors and knee extensors will be conducted using the BIODEX ergometer. The highest value of three consecutive maximal strength testing efforts will be recorded. Specific methods for testing can be found in the BIODEX manual of operations. Quantitative hand grip strength will be assessed using the CiTEC handheld ergometer, again recording the highest value of three consecutive tests for each hand. Methods for testing can be found in the CiTEC manual of operations.

- **Pulmonary Function Tests (30 minutes)**

  Standard PFTs will be done using the Renaissance II spirometer. Spirometry has been used extensively as a measure of respiratory function. PFTs include maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), forced vital capacity (FVC), forced expiratory volume 1 (FEV1), peak expiratory flow (PEF), maximum ventilatory volume (MVV) and peak cough expiratory flow (PCEF).

- **Timed Function Testing (5 minutes)**

  Timed Function Testing has frequently been used to assess functional abilities in three important areas: climbing four standard stairs and walking/running 10 meters. Each of these skills is timed to provide a quantitative measure of function. Specific methods are outlined in Florence et al [88].

- Multifrequency Bioimpedence Assessment (MFBIA)

  Participants will undergo multifrequency bioimpedence assessment (MFBIA) testing using a Xitron model 4000 MFBIA system to painlessly and non-invasively assess the amount of total body water,

intracellular water, extracellular water and fat-free mass. One electrode is placed to bisect the ulnar head, and the other electrode is placed just behind the middle finger. On the ankle, one electrode is placed to bisect the medial malleolus, and the other electrode is placed just behind the middle toe. Impedence is measured between upper and lower extremity electrodes.

- Echocardiogram.

  Participants will undergo echocardiogram with collection of left ventricular (LV) circumferential and longitudinal strain, LV shortening fraction, LV ejection fraction, end systolic volume, and end diastolic volume as described by [89].

9 ASSESSMENT OF SAFETY

9.1 Safety Parameters

Safety parameters for this study will include:

- Review of Systems
- Review of Adverse Events
- Laboratory Assessments: Concurrent with peripheral blood draws listed in the efficacy section and according to the study visit procedure chart, participants will provide blood samples for standard clinical safety testing. Safety panels will include:
  - CBC/Diff
  - PT/PTT
  - CPK
  - Total cholesterol
  - Albumin
  - Total Bilirubin
  - Conjugated Bilirubin
  - GGT
  - ALT/AST
  - Alkaline Phosphatase
  - Sodium
  - Potassium
  - CO2
  - Chloride
  - Calcium
  - BUN
  - Serum Creatinine
  - Serum Glucose
  - Serum Total Protein
  - Fasting lipid profile
  - BNP
- EKG
- Review of Medical History
- Review of Medical and Surgical Events
- Physical and Neurological Exam
- Review of Medication History
- Review of Current Medications and Therapies
- Review of any Adverse Events
- Collection of Vital Signs
- Collection of Height & Weight

9.2 Procedures for Reporting Adverse Events

Adverse events must be recorded in the study source documentation and eCRFs. All events occurring after the informed consent is signed must be reported regardless of whether or not they are believed to be

related to the study drugs or procedures. During study participation, adverse events should be followed by the study site principal investigator until resolved. All adverse events are graded according to the CTCAE.

9.3 Data and Safety Monitoring Board / Medical Monitor

This study will not employ a Data and Safety Monitoring Board (DSMB) but adverse events will be reviewed by an appointed Medical Monitor who is otherwise not affiliated with the study.

In the event of a SAE, the Medical Monitor will be notified within 24 hours of notice of the event to the Coordinating Center, and details of the event will be provided for review at that time. Additional follow-up information will be provided to the Medical Monitor within 15 days of the initial event. Summaries of all other reported adverse events will be reviewed by the Medical Monitor on a quarterly basis.

9.4 Duration of Participation / Follow-Up

Participants will participate in the study for a period of approximately 48-50 weeks from the date of enrollment (including screening).

9.5 Trial Stopping Rules or Discontinuation Criteria

Participants may withdraw from the study at any time without prejudice. Study investigators may withdraw participants at any time for reasons such as:

- Inability to comply with study protocol
- SAEs
- Non-tolerable or non-manageable adverse events (AE)
- Severe or sustained unexplained laboratory abnormalities
- Recommendation of suspension of the study by the Medical Monitor

10 HUMAN PARTICIPANTS

a. Characteristics of the Study Population

Ambulatory male adults with a confirmed diagnosis of BMD, 18 years to 70 years, will be recruited.

b. Participation of Children, Women and Minority Populations

BMD is an X-linked recessive disease affecting only males. However, female carriers of the disease can be symptomatic due to skewed X-inactivation. We have opted to study the most commonly affected population, males, to ensure patient homogeneity.

c. Sources of Research Material from Living Participants

Study data and blood samples collected will only be for research use or monitoring of study safety.

d. Recruitment of Participants

Participants will be recruited through the clinics of participating investigators, advertising and medical record screening in participating clinics. This trial will also be listed and updated on www.clinicaltrials.gov.

i. Advertising

Any local advertising through newsletters or muscular dystrophy-associated organizations' mailings will be submitted to the local institutional review board (IRB) that provides overview of this project.

ii. Screening

Patients for this study will be identified from the prior study cohort enrolled in the UCD0113 proof of concept BMD epicatechin study. Personnel who review existing patient medical records must be designated by the study site's principal investigator and must be an employee of that institution. A list of those personnel must be supplied to the institution's IRB.

e. Informed Consent/Assent and Ethical Considerations

Informed consent/assent must be documented for each participant. The date and time of the consent/assent must be prior to the initiation of any study-related tests or procedures, including diagnostics

that might be required to confirm a participant's study eligibility. The consent/assent form should supplement, not replace dialogue between the study principal investigator and the patient.

f. Retention of Participants

Care of each participant will be supervised by the participating site's principal investigator and study coordinator, who will schedule all visits and assessments.

If a participant is non-compliant, efforts will be made by site staff to ensure participant compliance, including participant/family teaching and increased frequency of phone contact to reiterate necessary information. If compliance does not improve, the investigator may decide to withdraw the participant from the trial. Any withdrawn participant will complete a follow-up visit along with an overview of systems and any adverse events that were noted during their study participation. Any ongoing adverse events will be monitored for 30 days after the participant's follow-up/withdrawal visit.

g. Potential Risks

i. Risks of Epicatechin

Animal and human safety studies suggest that (-)-epicatechin at doses of 1-2 mg/kg should be safe and well tolerated. 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 [71]. For the human dosing plan of 1 mg/kg, this provides a safety margin relative to the rat NOAEL of at least 85X. The dosing plan is also supported by published human studies, in which (-)-epicatechin doses of 1 and 2 mg/kg were administered with no adverse events were reported [42]. Potential risks based on the biological activities of (-)-epicatechin include:

Hypotension Given the reported effects of (-)-epicatechin on blood vessels, it is reasonable to assume that a potential risk may be associated with vasodilation. Of note is the fact that so far, no blood pressure reducing effects by (-)-epicatechin have been reported in normal subjects [90]. With cocoa based studies, blood pressure reducing effects are only reported in humans that have high blood pressure [90]. There is the possibility that patients undergoing pharmacologic treatment for high blood pressure if given (-)-epicatechin may develop hypotension through additive or synergistic effects.

Migraines The facilitation of migraines has been associated with the action of vasoactive substances [91]. The consumption of cocoa products has been reported to be associated with increased likelihood of migraine development [92, 93]. Thus, it is reasonable to surmise that (-)-epicatechin may increase the chances for migraine development in susceptible individuals.

Bleeding Anti-clotting like effects have been described for multiple members of the flavonoid family including (-)-epicatechin [94]. There is no report as to the effects that (-)-epicatechin per se may have on phenomena such as platelet aggregation or clotting times. There is a limited number of reports on the effects of cocoa on these endpoints. In general, they report effects on platelet aggregate
As with any compound, hypersensitivity reactions may occur, although no such reactions have been seen to date.

Contraindications and Warnings No contraindications are known. However, due to the possibility that (-)-epicatechin may interact with drugs with known antiplatelet effects to potentiate their activity, participants who are currently on long-term therapy with any such agents will be excluded from the study.

Overdose Neither the effects of overdose of (-)-epicatechin nor an antidote to overdose are known. The intraperitoneal (-)-epicatechin median lethal dose (LD_{50}) reported for mice is 1000 mg/kg as stated in the MSDS provided by suppliers such as Sigma-Aldrich.

Pregnancy and Lactation In a developmental toxicity study in pregnant rats using a green tea extract, the no-observable-adverse-effect level (NOAEL) corresponded to 100 mg (-)-epicatechin/kg (the highest dose tested)[72]. No information is available on levels of (-)-epicatechin in breast milk.Risks of

ii. Risks of Blood Tests

The risks of blood drawing include soreness or bruising at the site of the needle. A local numbing cream (EMLA) may be applied to the area at the participant's request. There are no side effects associated with the use of this cream. Rarely, a more serious injury, such as hematoma (bleeding under skin) or infection may develop.

iii. Risks of QMT Strength Testing

QMT will be done at each visit to measure strength in the participant's muscles. At this time, there are no known risks associated with functional evaluation or muscle strength testing methods used in this protocol. However, the participant may experience mild muscle soreness the day after muscle testing.

iv. Risks of PFT

These tests may cause dizziness and lightheadedness during and shortly after the test.

v. Risks of EKG

The EKG has no known risks.

vi. Risks of Functional Evaluation and Muscle Strength Testing

At this time, there are no known risks associated with functional evaluation or muscle strength testing methods used in this protocol. However, the participant may experience mild muscle soreness the day after muscle testing.

vii. Risks of DEXA

DEXA scans involve exposure to a very small amount of radiation (<1.0mrem). DEXA is contraindicated in pregnancy (not applicable in this project).

viii. Risks of Multi-Frequency Bioimpedence Assessment (MFBIA)

There are no known risks of MFBIA testing.

ix. Risks of Upper Extremity Range of Motion Evaluation

There are no known risks of upper extremity range of motion evaluation.

h. Procedures for Minimizing Risks

Confidentiality of medical information will be maintained throughout the study. Participants will be assigned identification numbers that will be used on all case report forms. No personally identifiable information will be released beyond the Study Coordinating Center without the participant's prior written consent. Data entered into electronic case report forms will be handled by the RedCap online CRF system and managed in compliance with FDA privacy and data retention standards for electronic clinical research data collection.

Safety data recorded during the conduct of this study will be transmitted directly to the Medical Monitor through the RedCap system. Data will be collected at each study visit and over the phone (family reported AEs) between study visits. Both AEs and SAEs will be reviewed by the Medical Monitor. AEs will be reviewed on a quarterly basis by the Medical Monitor.

i. Justification of Risks to Participants

Due to the low toxicity profiles of (-)-epicatechin, risks to subjects associated with participation in this study are less than or similar to standard clinical interventions in patients with BMD.

j. Benefits

The intended benefits outweigh the risks of using the study medication and study procedures in BMD patients. The participants may experience an increase in muscle strength or a delay in strength decline. The participants will receive additional care during study participation including muscle strength training, contracture measurements and spirometry during each study visit. Medical and adverse event history will be closely monitored. The additional medical monitoring allows for increased interaction with medical staff above expected routine clinical care. The increased monitoring of safety labs will also be performed.

It is possible the participants will not experience any direct benefit as a result of their study participation. However, the data collected during this trial may provide information that will benefit the scientific community as well as other individuals with BMD

k. Financial Considerations

No financial compensation will be given to participants or their families for participation in this trial other than minor assistance with transportation expenses (parking, etc.).

11 STATISTICS

Presented herein is the overall analysis plan. The main criterion for success of the study will be presence of one or more biologic or strength and performance outcome measures (Aims 1 and 2) that yield a response magnitude that allows for sufficient power in a Phase II B study with a sample size of 30 individuals. Analysis, employing both statistical and graphical presentations of data, will proceed from descriptions and simple comparisons to multiple variable models. This will help to ensure proper understanding of the data at each level before proceeding to the next. Descriptive analysis of means and standard deviations or frequencies and proportions will characterize study participants overall as well as evaluate assumptions of normality and homoscedasticity. Any significant departures from these assumptions especially for the measurement variables will lead to normalizing or variance stabilizing transformations or, in the unlikely event these are not successful, to conversion to ranks. Level of statistical significance is set at <0.05. Simple comparative analyses will be used to assess and understand simple relationships, including defining first order interactions. We will compare continuous variables across groups using the Student's t-test and analysis of variance. We will compare differences between groups using Chi Square and Fisher's Exact tests. We will compare simple relationships between variable using parametric or nonparametric correlations as appropriate. Power calculations will be based on simple between-group comparisons of means for treated / non-treated individuals at specified time periods. However, we will also explore complex relationships between variables and differences between groups using a combination of linear regression and longitudinal multivariate mixed model approaches (using xtreg or xtmixed in Stata12). These procedures will allow us to account for fixed and random effects as well as correlation between observations when we introduce repeated measures on the same individuals. Pilot data will be used to determine estimates of minimally important clinical difference (MCID) for each measure (defined as 1/3 S.D. for the population). Safety data will be compiled as tables of frequency and severity of adverse events by body system.

12 DATA COLLECTION

12.1 Data Management System

A web-based clinical data entry system, RedCap is being used for electronic case report form (eCRF) data collection. The data management system complies with all federal regulations pursuant to 12 CFR Part 11.

12.2 Data Quality Control and Quality Assurance

The RedCap System will provide validity checks on participant and visit identifier fields, as they are entered, to ensure that the visit is unique and appropriately timed according to protocol criteria. A secure web-based 'smart' eCRF system will detect some inaccuracies in data entry immediately to alert site study staff prior to data submission.

12.3 Security and Backups

To ensure patient confidentiality, no patient identifiers are entered into this system. Sequential study numbers are assigned to all participants. All computers are password protected and accessible only to

study personnel. All data entered into the RedCap System are copied by the UC Davis CTSC Data Coordinating Center to a secure back-up server at another site several times per day.

12.4 Data Monitoring

Over the course of the study, the Study Chair, Project Manager, biostatisticians and Medical Monitor will require access to the entire study dataset. This may be for the purposes of monitoring a specific site's data, performing quality control, performing periodic data analysis or for safety or efficacy monitoring. The above named individuals will have read-only access to the study data via the RedCap web interface.

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