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## **The Effects of Glutamine and Fish Oil Supplementation on Exercise Tolerance in Heart Failure**

### **I. Study Purpose and Rationale**

Hypothesis: Supplementation of glutamine or fish oil for 30 days will improve exercise tolerance in patients with chronic heart failure.

#### Rationale:

With its increasing prevalence throughout the world, heart failure continues to be associated with high morbidity and mortality. There is a progressive development of metabolic abnormalities, inflammation, and atrophy in the myocardium and skeletal muscle. The improvement in functional capacity as defined by exercise tolerance is essential for prolonged survival and better quality of life for these patients. Therapeutic management addressed at improving peripheral function is lacking. Therefore, nutritional approaches with dietary supplementation in addition to current therapies are particularly appealing as they are novel and mechanistically different. Glutamine is of particular interest because it is proposed to replete intermediates in the TCA (tricarboxylic acid) cycle and perhaps activate the suppressed oxidative metabolism in heart failure. Fish oil is of interest because of its activation of lipolysis its anti-inflammatory properties; it has proven to be beneficial for central cardiac dysfunction but studies on its effect in skeletal muscle must be pursued.

#### Background:

Heart failure (HF) affects 5 million Americans with an incidence of 10 per 1000 over the age of 65 [1]. Despite considerable medical progress, the annual mortality is still 10% [2]. Heart failure is a complex syndrome due to an initial injury followed by a progressive decline in cardiac output that eventually curtails systemic metabolic needs. It is characterized by symptoms of dyspnea, peripheral edema, and exercise intolerance. Medications such as angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists,  $\beta$ -adrenergic receptor antagonists, and aldosterone receptor antagonists suppress the neurohormonal activation seen in HF and diuretics, digoxin, and inotropic agents treat fluid overload and perturbed hemodynamics.

Exercise intolerance, marked by exertional dyspnea, is the major morbidity of heart failure. The Fick equation is used to calculate the maximum oxygen consumption by taking the product of cardiac output and the peripheral A-VO<sub>2</sub> difference [3]. The central cardiac factor is inherently limited because the heart is in failure and limited changes can be made to heart rate and contractility. Therefore, functional capacity is best modulated by peripheral tissue metabolism. In addition, it has been shown that there is little correlation between central hemodynamics and exercise intolerance [3]. There are

also no improvements in functional capacity when leg blood flow is increased; there remains an early presence of lactate and anaerobic metabolism during exercise despite this correction in blood flow [3].

### *Normal Metabolism*

The function and morphology of both myocardium and skeletal muscle in heart failure is directly linked to cellular metabolism and the balance between anabolism and catabolism. In the normal heart, greater than 95% of the ATP formation comes from oxidative phosphorylation in the mitochondria [4]. The fuel for mitochondrial respiration is generated from the tricarboxylic acid (TCA) cycle,  $\beta$ -oxidation of fatty acids, and glycolysis and carried to the electron transport chain via equivalents of NADH and FADH<sub>2</sub>. The fundamental metabolism of glucose and fatty acids is similar in the myocardium and the skeletal muscle. Either the GLUT1 or GLUT4 transporter brings glucose into the myocyte. Glucose is also derived from breakdown of intracellular stores of glycogen. Pyruvate is the key irreversible step in carbohydrate oxidation and is catalyzed by pyruvate dehydrogenase (PDH). PDH is inactivated by phosphorylation by specific PDK kinases, of which PDK4 is most important. Fatty acids enter the myocyte by passive diffusion or via protein-mediated transport mainly by FAT/CD36 and are further esterified to fatty acyl-CoAs by acyl-CoA synthetases. Carnitine palmitoyl transferases (CPT) transport long chain fatty acids into the mitochondrial matrix for  $\beta$ -oxidation. 60-90% of the acetyl-CoA comes from  $\beta$ -oxidation of fatty acids and 10-40% comes from the oxidation of pyruvate, which originates in glycolysis and lactate production [4].

### *Metabolic Regulation*

Metabolism is determined by the rate of flux, expression, activity, and characteristics of regulatory proteins and the tissue content of regulatory metabolites [4]. In 1963, Philip Randle proposed that there is a flux to fuel utilization such that there is competition between glucose and fatty acids for its oxidative metabolism [5]. There are multiple points of regulation in which enzymes are regulated by preferential substrates. For example, increased acetyl-CoA inhibits PDH, citrate inhibits phosphofructokinase (PFK-1) and glucose-6-phosphate inhibits hexokinase, all indicating decreased glucose utilization. Glycolysis generates pyruvate and induces inhibition of PDK, leading to increased acetyl-CoA and citrate production. Citrate can escape into the cytosol, be converted to acetyl-CoA and malonyl-CoA, which is a potent inhibitor of CPT.

### *Metabolic Dysfunction in Heart Failure*

Animal models suggest that in early heart failure there is an increase in fatty acid (FA) oxidation, but then a dramatic switch occurs in severe or decompensated HF. In humans, changes in myocardial metabolism also occur late in the development of HF [4]. Cardiac metabolism shifts from fatty acid oxidation to oxidation of glycogen, lactate, and glucose [6]. The failing human heart regresses to the fetal gene program by decreasing expression of CPT mRNA, MCAD (medium chain acyl-CoA dehydrogenase) mRNA, and citrate synthase activity while increasing PDK mRNA [7]. In the failing heart, there are also defects in mitochondrial function in which reduced activity of electron transport chain (ETC) complexes lead to decreased oxidative metabolism. There is a

downregulation of fatty acid (FA) oxidation genes triggered by defects in the ETC as well as a reduction in PPAR-alpha expression [8]. Di Lisa et.al. showed that the maximal activity of PDH was significantly lower in two murine models of cardiomyopathy suggesting that the complete oxidation of fatty acids would be impaired [9]. Furthermore, Bersin et.al. showed that intravenous dichloroacetate, a PDH kinase inhibitor, improved cardiac function by increasing pyruvate oxidation [10].

It is proposed that the same metabolic phenotype that occurs in the heart occurs in the skeletal muscle in heart failure. The mitochondrial volume density, cristae surface density, and cytochrome oxidase activity are substantially reduced in patients with severe HF [11]. Rats with heart failure have an increase in type II glycolytic fibers, decreased citrate synthase activity, and reduced cytochrome C oxidase III activity [12]. Studies on the skeletal muscle metabolism in heart failure are sparse, but the trend for decreased or unchanged oxidative metabolism and increased glycolysis is as present as it is in the heart. The switch for this change in substrate utilization is unknown.

### *Warburg Hypothesis*

In 1956, Otto Warburg found that in proliferating ascites tumor cells that glycolytic flux was high and instead of oxidizing glucose, it was converted to lactate even when oxygen was present; this “aerobic glycolysis” has now become the Warburg hypothesis. Although the ATP production per molecule is low using glucose, if the glycolytic flux is high enough it can exceed the amount of ATP produced from oxidative phosphorylation [13]. During cell proliferation as in cancer, it is possible that the high rate of glycolysis conserves intermediates of the TCA cycle for biosynthetic pathways (lipids, proteins, and nucleic acids) for growth [13].

### *Anaplerosis*

Anaplerotic reactions are those that provide intermediates into the TCA cycle. Enzymes involved in anaplerosis are pyruvate carboxykinase (PEPCK), pyruvate carboxylase (PC), malic enzyme (ME), purine nucleotide cycle (PNC), alanine aminotransferase (ALAT), glutamate dehydrogenase, and glutaminase [14]. The PNC results in formation of fumarate, but human studies do not support a role for PNC to increase overall flux of TCA intermediates (TCAi); during exercise, it has been shown that PNC is unlikely to have a major role in the skeletal muscle [15]. In addition, PC, PEPCK, and ME are not significant players in the process of anaplerosis [15]. Acetyl-CoA is not the regulating factor in the TCA cycle [16]. In fact, in the rat heart substrates that supply only acetyl-CoA do not improve contractile performance whereas other anaplerotic pathways do [17].

During exercise in skeletal muscle, ALAT activity increases flux into the TCA cycle through pyruvate and 2-oxyglutarate formation [18]. In an isolated working rat heart, activity of 2-oxoglutarate dehydrogenase provides a quantitative index of TCA flux whereas no correlation was observed between TCA flux and succinate dehydrogenase or citrate synthase [19]. Further, after 10 minutes of exercise, glutamine infusion increased TCA intermediates whereas ornithine  $\alpha$ -ketoglutarate did not [14]. On the other hand, Constantin et.al. showed that in skeletal muscle DCA (dichloroacetate)

infusion which activates PDC before exercise improves exercise capacity in humans by decreasing lactate production and increasing the muscle TCAi pool due to increased pyruvate availability [20]. In a heart oxidizing acetoacetate, there is inhibition of 2-oxoglutarate dehydrogenase noted by an increase in 2-oxoglutarate and glutamate [21] and contractility is maintained by carboxylation of pyruvate allowing it to enter the TCA cycle [17]. Hermann et.al. infused pyruvate (150 mmol/L, supraphysiological concentration) for 15 minutes two times into the left main coronary arteries of 8 patients with dilated cardiomyopathy and showed an inotropic effect: increase in cardiac index, stroke volume, and decrease in pulmonary capillary wedge pressure [22].

Studies concerning anaplerotic reactions and substrates are not consistent in terms of defining which pathways are most essential, but from those that are present, both glutamate and pyruvate play a major role. It can also be stated that anaplerotic flux may be more important than TCA content or the total concentration of any of the TCAi in determining the overall TCA flux [15].

### *Cachexia*

Cachexia is weight loss with a preferential loss of lean tissue mass, a pattern that is similar across chronic diseases, such as cancer and AIDS [23]. In HF, the metabolic and functional derangements usually precede the loss of muscle mass. At this end stage, there is increased net protein degradation leading to skeletal muscle wasting. In early stages of HF, there is increased atrophy which correlates with reduced expression of IGF-1, activation of systemic and local markers of inflammation such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and iNOS, and increased oxidative stress [24]. A major player catabolism is the ubiquitin-proteasome system and associated atrogenes atrogen-1 and MuRF-1.

### *Glutamine*

Glutamine is the most abundant amino acid (AA) in mammals and along with glucose, accounts for the majority of the carbon and nitrogen metabolism in the cell [25]. The significant tissue producers of glutamine are skeletal muscle and liver [26]. Skeletal muscle glutamine concentration is approximately 20 mmol/kg net weight and consists of 60% of the total free AA pool in skeletal muscle [27]; muscle releases 50 mmol/hour glutamine in the fed state [26]. Equally, the plasma concentration of glutamate is 64  $\mu$ mol/L whereas in the skeletal muscle it is 4000  $\mu$ mol/kg wet weight [28]. It is a nonessential AA that is involved in gluconeogenesis, acid base regulation, and synthesis of glutamate, nucleotide bases, and the antioxidant glutathione [29].

Amino acids are digested and broken down further into di- and tripeptides before being absorbed across sodium-dependent amino acid transporters. There is an active dicarboxic transporter for glutamate in skeletal muscle, X<sup>-</sup>ag [28]. Protein breakdown will also contribute to the intracellular glutamate concentration. The predominant anaplerotic reaction in exercising human muscle is ALAT, an enzyme that catalyzes the conversion between pyruvate and glutamate and alanine and  $\alpha$ -ketoglutarate [15]. In the rat, levels of glutamate and glutamine are 3 to 5 fold higher in the slow-twitch soleus muscles than in the fast twitch plantaris and gastrocnemius muscles [28].

Most studies with glutamine supplementation have been completed in acute settings where it is infused and testing is done within minutes or after a bout of exercise. The data for these experiments is not clear. At the start of exercise, intramuscular glutamate decreases 60% which by <sup>13</sup>C NMR spectroscopy reveals increased conversion to alanine and lactate [30]. Some believe that early metabolic support can improve the recovery of an ischemic heart [31]. When rats were induced to have myocardial infarction, only those reperfused with glutamine, versus aspartate and glutamate, showed a significant recovery of cardiac output, prevented a fall in the ATP/ADP ratio, and decreased GSSG concentrations [32]. Others argue that ingestion of glutamine before exercise augments the TCAi pool but does not increase TCA flux nor extend endurance capacity [33]. Right after exercise, glutamine infusion results in muscle glutamine increase of only 15% whereas plasma glutamine increases 70% [14]. In a group of male human patients with chronic stable angina and with positive Bruce exercise test given 330 ml carbonated water enriched with glutamine (80 mg/kg) prior to exercise. The only finding was a delayed onset of 1 mm ST depression [32].

Amino acid intake in elderly sedentary patients results in increased ambulatory capacity (increased distance in a 6 minute walk test) and maximal isometric muscle strength [34]. Aquilani et.al. gave amino acid (AA) supplementation (4 grams twice daily) for 30 days to elderly with HF and showed improved exercise capacity, reduced circulatory dysfunction, and increased peripheral oxygen availability [35]. There was an increase in peak VO<sub>2</sub>; the recovery time was quicker and the time at peak VO<sub>2</sub> was less in the AA group. In a group of subjects with Type 2 DM (at risk for HF) and no coronary artery disease (CAD) history, 12 grams AA/day were administered for 12 weeks in a randomized single-blind crossover study and results showed that exercise LVEF was higher during AA intake [36]. Others argue that ingestion of glutamine before exercise augments the TCAi pool but does not increase TCA flux nor extend endurance capacity [33].

Glutamine increases the balance of nitrogen, attenuates skeletal muscle proteolysis, and is essential for immune system function [37]. In 78 patients undergoing cardiac bypass surgery, subjects given high dose glutamine (0.5g/kg/day), there was no difference in T-cell inflammatory response after surgery, no changes in circulation, ventilation time, and ICU length of stay (LOS) [38]. 22 patients undergoing CABG surgery after aortic cross-clamp release, were given 125 mmol/l glutamate for 1 hour, blood flow increased and resistance decreased without significant effect on cardiac substrate metabolism rather more of a vasodilatory effect on hemodynamics [39]. Glutamine acts in gluconeogenesis by saving phosphocreatine deposits and glycogen in muscle fibers, especially type 1 fibers [40]. Soccer players took either 50 grams maltodextrin plus 3.5 g glutamine in 250 ml water or just 50 g maltodextrin (control) 30 minutes before exercise and the former group experienced less fatigue and covered a greater mean distance [40]

#### *Fish Oil*

Omega-3 fatty acids are essential because they cannot be synthesized de novo due to the lack of synthetic enzymes in fatty acid synthesis. The most widely available form is

alpha-linoleic acid (ALA), found in vegetable oils. However, very little of this is converted into eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) (1-5% and 0.1%, respectively) [41]. Fish oil in the human diet is primarily obtained by eating fish such as herring, mackerel, salmon, and sardines or through supplements [42]. Most clinical supplements are a mixture of EPA and DHA and there are few data studying each of these components separately. The AHA has recommended 1 g/day of a combination of EPA and DHA for those documented with coronary heart disease.

The GISSI-HF trial randomized 7,046 chronic heart failure patients to placebo and n-3 polyunsaturated fatty acids and showed a small beneficial advantage in mortality and admission to the hospital for cardiovascular reasons [43]. Extensive evidence has shown that dietary omega-3 polyunsaturated fatty acids are cardioprotective in terms of coronary artery disease and sudden cardiac death [41]. In the Diet and Reinfarction Trial (DART), subjects eating fatty fish had a 29% reduction in 2-year all cause mortality [44]. The JELIS trial showed a 19% reduction in incidence of CAD in Japanese patients taking supplements of EPA after being followed for an average of 4.6 years [45].

A proposed mechanism for cardioprotection is improved mitochondrial function and efficient ATP production, but the exact mechanism is unknown. Fish oil also reduces circulating pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 and IL-6 versus control using low dose 0.85 g of PUFA per day (EPA and DHA in ratio of 1:1.2). These anti-inflammatory effects contribute to an anti-catabolism effect.

In healthy subjects, there was no difference in peak  $VO_2$ . 16 well trained cyclists randomized to either 8 g/day fish oil capsules (0.8 g EPA and 2.4 g DHA) or olive oil capsules (control) for 8 weeks and results showed that there was no difference in peak  $VO_2$ , maximum workload, and time to voluntary fatigue [46]. 8 healthy male volunteers in a crossover study with and without supplementation of 7.2g/day fish oil (6 capsules of 1.2 g /day, 1.1 g EPA and 0.7 g DHA) and a 6 week washout period for 14 days of treatment showed that there was no change in maximum  $VO_2$  and energy efficiency [47].

The combination of high protein, leucine and fish oil and not each one alone resulted in a reduction in the loss of body mass and improved muscle function in a murine model of cancer cachexia [48]. 85% of pancreatic cancer patients experience cachexia [49]. Patients with pancreatic cancer received a nutritional supplement enriched with fish oil (610 kcal, 32.2 g protein, 2.2 g EPA, 0.96 g DHA) for 3 weeks and results showed a weight gain, decreased production of IL-6 and decreased proportion of patients excreting proteolysis inducing factor [50].

## **II. Study Design**

This will be a prospective, randomized double-blind study design. Patients will be randomized to three groups: placebo alone (2 subgroups with one getting placebo powder and the other placebo capsules), glutamine alone, and fish oil alone for the duration of 30 days. The outcomes of interest in this study are the changes in peak  $VO_2$ ,

maximum muscle strength in the dominant leg, duration of exercise until onset of fatigue, and exercise distance based on the six minute walking test at baseline and after treatment.

Having a fourth where the treatment was a combination of glutamine and fish oil was considered, but the question of compliance was of concern. Neither the amino acid supplement nor the fish oil capsules are palatable so taking both supplements would not appeal to the subjects. In addition, there is concern over the gastrointestinal side effects, since both supplements can result in diarrhea or steatorrhea and both would be used at higher doses for optimal effect.

Comparing placebo to a combined glutamine and fish oil supplementation group only would protect against the unnecessary costs in time and money if both interventions show no significant differences in the outcomes measured. The problem that one crosses is that although the mechanisms of action appear to be different, it is possible that interference can occur and cause no effect when the single agents do have an effect.

A crossover study design in which a desired duration of treatment was 30 days would require a longer period of study for the enrolled patients. In addition to the periods for each treatment, washout periods between each would also have to be implemented. Since this is a preliminary study assessing for change, a three arm placebo controlled design is optimal for time and cost.

### **III. Statistical Analysis**

Patient characteristics will be compared between patients by using the  $\chi^2$  test for categorical variables and t-test for continuous variables.

#### Peak VO<sub>2</sub>

In a group of 16 patients with NYHA Class II or III and ejection fraction of 26%, the mean peak VO<sub>2</sub> was 16.3 ml/kg/min [51]. In the HF-ACTION trial, baseline peak VO<sub>2</sub> for chronic heart failure patients was 17.5 with a range from 16 to 20 [52]. In the same trial, patients with systolic HF showed a baseline of 14.9 ± 4.7 ml/kg/min [53]. For NYHA Class II HF patients, the mean peak VO<sub>2</sub> is 16.1 ± 4.6 ml/kg/min versus 13.0 ± 4.2 for NYHA Class III/IV patients [54]. Exercise training increases peak VO<sub>2</sub> by 26% [55]. We will use a baseline of 17.5 ml/kg/min. Based on the AHA Statement which reviewed the effects of exercise in heart failure in 2003, an average increase of 15% occurred for VO<sub>2</sub> in a duration of less than 8 weeks [56].

Amino acid intake in elderly sedentary patients results in increased ambulatory capacity (increased distance in 6 minute walk test) and maximal isometric muscle strength [34]. Aquilani et.al. gave amino acid supplementation (4 g bid) for 30 days to elderly with HF and showed improved exercise capacity; the peak VO<sub>2</sub> increased by 12.7% [35]. The effect of fish oil on peak VO<sub>2</sub> is not known, but we predict that it will be lower than that of glutamine. A clinically significant change in peak VO<sub>2</sub> would be approximately 10%.

An unpaired t-test will be used to compare the change in  $VO_2$  in the group with glutamine and the change in  $VO_2$  in the control group. I expect the glutamine group to have an increase around 10% in  $VO_2$ , which would be approximately  $2 \text{ ml/kg/min} \pm 0.5 \text{ ml/kg/min}$  using 17.5 as a baseline approximation. The sample size needed at a power of 0.80 and a significance level of 0.01 is less than six subjects. Since the effect of fish oil has not been studied in skeletal muscle, I would be more conservative in calculating sample size for this group. To expect a change of 5% in the  $VO_2$  in the fish oil group, a 5% change is calculated as a change of approximately  $1 \text{ ml/kg/min} \pm 0.5 \text{ ml/kg/min}$ . The sample size needed at a power of 0.80 and a significance level of 0.01 is eight subjects. If I used 15 people in each group, with a standard deviation of 0.5 ml/kg/min, I would be able to detect a difference of 0.67 with 80% power at a significance level of 0.01.

To aim for a larger number in each group is important because I would expect some subjects to drop out from the study. In the GISSI-HF trial, 3% discontinued the treatment because of adverse effects, mostly GI disturbances, in both placebo and n-3 PUFA groups [43].

### Maximum Muscle Strength and Fatigability

In a cohort of 122 patients with a mean ejection fraction (EF%) of 22%, the baseline isokinetic torque was  $79 \pm 30$  and  $132 \pm 47 \text{ Nm} \times 100/\text{kg}$  for the knee flexor and knee extensor, respectively [57]. In a baseline exercise capacity measurement in chronic heart failure patients, quadriceps isometric strength was  $444.9 \pm 129.6 \text{ N}$ , quadriceps peak torque was  $123.6 \pm 30.2 \text{ Nm}$ , and hamstrings peak torque was  $53.6 \pm 15.6 \text{ Nm}$  [58]. Combined endurance and strength training over a two month period improved peak torque of the extensor significantly (10%) and peak flexor torque by 7% (not significant) [59]. Using a sample size of 15 and a standard deviation of 30, power of 80% and significance level of 0.05, I could detect a minimum difference of 32 Nm in quadriceps peak torque between placebo and treatment groups. Using a sample size of 15 and a standard deviation of 16, power of 80% and significance level of 0.05, I could detect a minimum difference of 17 Nm in hamstring peak torque between placebo and treatment groups. Both values are greater than what has been shown with exercise.

In a study to assess the effect of clenbuterol on skeletal muscle function, the baseline exercise time before fatigue for heart failure patients was  $11.9 \pm 3.9$  minutes.

### Six Minute Walk Test (6MWT)

Mean distance in two studies was 433 and 455 meters in NYHA II/III patients [51]. In patients with left ventricular dysfunction marked by an ejection fraction less than 40% had a change in  $281.2 \pm 264.7$  meters (m) after a 14 week exercise protocol [60]. In the HF-ACTION trial, baseline 6MWT for chronic heart failure patients was 414 m with a range from 357 to 472 m [52]. In the same trial, patients with systolic HF showed a baseline of  $365 \pm 105 \text{ m}$  [53]. Using a sample size of 15 and a standard deviation of 200, power of 80% and significance level of 0.05, I could detect a minimum difference of 212 between placebo and treatment groups.



## **IV. Study Procedures**

Baseline characteristics that will be obtained through interview and chart review include age, gender, etiology of heart failure (dilated cardiomyopathy, coronary ischemic, valvular, and other), NYHA functional class, heart failure duration, past medical history, body mass index (BMI), current medications, smoking history, and alcohol and drug history.

Echocardiography will be done to assess the ejection fraction (EF%) of the subject before enrollment in the study and after treatment. The subject's heart rate and blood pressure will be recorded at the initial visit and after treatment.

Subject's blood will be drawn at the initial visit and after treatment. The plasma levels of glutamine and glutamate will be assessed.

### Cardiopulmonary Exercise Testing

Treadmill exercise testing in association with air-gas-exchange is an optimal gauge for functional capacity. The motor-driven treadmill will serve as the exercise stimulus. Graded exercise testing will be done with a modified Naughton protocol. During the exercise, the subject will keep a lightweight disposable pneumotach device in his or her mouth. The peak  $VO_2$  will be measured.

### Muscle Strength and Fatigability

Isokinetic CYBEX testing will be used to measure maximal muscle tension throughout a full range of motion in knee flexion and extension while the speed remains constant. Slow speeds of 30 to 60 degrees will be used to assess muscle strength [57]. Isometric strength will be taken as the average of three maximal voluntary contractions (MVCs) each lasting 3 seconds, with a minimum recovery period of 15 seconds between each. Once the maximum strength is quantified, the tension will be reduced to 80% of the maximal strength. The patient will be asked to make repeated movements of flexion and extension until he or she reaches exhaustion. The duration of exercise time before exhaustion will be used as a measure of fatigue.

### Six Minute Walk Test (6MWT)

A 25-meter course with a chair at each end will be marked in a corridor. Subjects will be asked to walk as fast as possible over a period of six minutes [61]. The test will be scored in rounded meters walked in 6 minutes. In the case patients are fatigued and need to sit, they will be allowed to. Symptoms experienced by the patient (angina, dyspnea, fatigue, dizziness, and syncope) will be recorded.

## **V. Study Drugs or Devices**

### Glutamine

Normal daily intake of glutamine from the diet is 3-6 g/day [29]. Glutamate itself cannot be used because transport of glutamate into skeletal muscle is poor [33]. It does not

reach the circulation as all the ingested glutamate is oxidized by the splanchnic bed on first pass [28]. Glutamine is easily converted to glutamate with glutaminase enzyme that is present in all cells. L-glutamine is available as a powder and in tablets or capsules of 250, 500, and 1000 mg. Doses of 0.65g/kg are maximally tolerated without abnormal plasma ammonia levels [29]. It is relatively unstable in solution and transport from the gut into the circulation is promoted by glucose and sodium.

The amount that will be used in this study will be 8 grams/day based on previous studies. The glutamine powder will be dissolved in water, perhaps mixed with a fixed amount of glucose or sodium to overcome issues with absorption, and given a flavor for ease of ingestion. The control group will also have a powder with the same adjuvants added, without the glutamine, and perhaps replaced with an isocaloric carbohydrate.

### Fish Oil

Fish oil can be taken orally as in the form of soft gel capsules or in a liquid drink with the lipid emulsion. The optimal dose and ratio of EPA to DHA is difficult to decipher. In the DART trial, 3 capsules of 0.5 grams of Maxepa were used (180 mg EPA and 120 mg DHA). In the GISSI-HF trial, subjects took one capsule/day of 850-882 mg EPA and DHA in a 1:1.2 ratio. In the JELIS study, subjects were given 2 capsules of 300 mg of greater than 98% EPA 3 times daily (a total of 1800 mg). Including smaller studies pertaining to cardiovascular health, 1-8 grams/day have been used. Two studies involving exercise capacity in healthy individuals showed that there was no effect on  $VO_2$  when given 8 grams or 7.2 grams of fish oil per day.

In pancreatic cachexia studies, the maximum dose tolerated is 0.3g/kg and the least amount showing an effect for weight changes is 12 grams. This would require a taking an equivalent of 12 capsules a day, which is quite high and would lead to noncompliance.

Most studies conclude that a greater difference in outcome may have been seen if a higher dose was used and therefore, encourage more trials with higher doses. Since heart failure patients are not healthy, I expect the differences to occur at lower concentrations than those used in healthy subjects. I would like to have the subjects take 3 capsules per day with EPA to DHA ratios the same as that used in the GISSI-HF trial. I would like to have a placebo group take capsules with olive oil or other lipid 3 times daily as well.

## **VI. Safety**

### Glutamine

The most common side effect for amino acid supplementation is dyspepsia.

### Fish Oil

The most common side effects are nausea, diarrhea, steatorrhea, abdominal cramps, and flatulence. Individuals also complain of the offensive taste and fishy belching. Since fish oil has been shown to decrease platelet aggregation and increase fibrinolysis, it is

important to know that there are no hemorrhagic complications up to a dose of 7g of combined DHA and EPA per day [62]. If the subject is on Coumadin or other blood thinners, especially because heart failure patients may have atrial fibrillation, careful dosing and analysis of any medication interactions are required. Regarding toxicity, no life threatening adverse events have been reported in the past studies.

If the patient has allergies or any side effects not mentioned, the supplement will be discontinued.

For exercise testing, emergency equipment and medical personnel will be in the immediate vicinity. Exercise tests will be stopped if the subject experiences dyspnea, fatigue, angina, arrhythmias, musculoskeletal complaints, or adverse changes in blood pressure.

## **VII. Study Questionnaires: N/A**

## **VIII. Study Subjects**

### Inclusion Criteria

- (1) Males and females, age  $\geq 55$  years
- (2) Left ventricular ejection fraction  $\leq 35\%$
- (3) Optimal therapy according to AHA/ACC and HFSA HF guidelines, including treatment with beta-blocker therapy (for at least 6 weeks), or have documented variation, including intolerance, contraindication, patient preference, or personal physician's judgment
- (4) Stable on standard heart failure medications

### Exclusion Criteria

- (1) Major cardiovascular event or procedure within the prior 6 weeks
- (2) Congenital heart disease, long QT syndrome, hypertrophic cardiomyopathy, or myocarditis as they increase the risk of adverse events from exercise testing
- (2) Dementia

## **IX. Recruitment of Subjects**

Patients will be recruited from the Heart Failure clinic at New York Presbyterian Hospital/Columbia University Medical Center (NYP/CUMC) campus. There are greater than 1000 heart failure patients at NYP/CUMC. The CUMC Heart Failure program includes men and women of a very diverse racial background. All enrollees will be over the age of 55 years, as this is the population most prone to heart failure and exercise intolerance.

Subjects will be screened at an outpatient visit after permission to approach the subject has been obtained from their personal physician. Subjects will be asked to sign consent by the study coordinator or physician in the study team. Subjects will be informed

verbally and in writing that participation is entirely voluntary and the decision to participate or not participate will in no way affect their care.

## **X. Confidentiality of Study Data**

All patient information will be kept confidential. Subjects will be identified by study identification numbers. All data will be kept in locked cabinets in locked closets. Access to electronic records will be restricted to research staff only.

Serious adverse events will be reported to the CUMC Institutional Review Board as per institutional guidelines within 24 hours.

## **XI. Conflict of Interest: NONE**

## **XII. Location of Study**

Patients will be screened and recruited at the heart failure outpatient clinic at CUMC. The location of the exercise testing will be determined upon arrival of the CYBEX exercise equipment.

## **XIII. Potential Risks**

Risks associated with the treatment arms are mostly the adverse effects mentioned above. The risks associated with exercise testing are small with an average rate of emergencies being 3.4 out of 10,000 tests. All tests will be performed by highly experienced personnel and risks will be addressed effectively by the research staff.

## **XIII. Potential Benefits**

The potential benefit of this study to the subject is improved exercise tolerance, which is one of the most prevalent problems in the syndrome of heart failure. Participation will certainly impact the future care of heart failure patients. The benefit to the researcher will be evidence to support a theory of anaplerosis and reprogramming of oxidative metabolism in skeletal muscle.

## **XIV. Pitfalls/Alternatives/Limitations**

If there is an improvement in exercise tolerance in either group, the mechanism of that improvement will be difficult to discern. With the glutamine group, we will not be able to distinguish the effect of protein synthesis versus anaplerosis and contribution to oxidative metabolism. With the fish oil group, we will not be able to distinguish if the improvement is due to a direct effect of fish oil on skeletal muscle abnormalities or a secondary effect after the known improvement in central hemodynamics.

## **XV. Compensation to Subjects**

There will be no monetary compensation for patients that complete the study.

#### **XVI. Costs to Subjects**

There will be no costs to the subjects, as this testing at initial and post-treatment visits will occur at times when the subjects are normally going to their outpatient office visits.

#### **XVII. Minors as Research Subjects: N/A**

#### **XVIII. Radiation or Radioactive Substances: N/A**

#### **XIX. References**

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