University of Arkansas for Medical Sciences (UAMS) Clinical Protocol

Study Title: Effects of 12 Weeks of Nutritional Therapy Interventions in Heart Failure

Principal Investigator: Gohar Azhar, M.D.
University of Arkansas for Medical Sciences
4301 West Markham St., Slot #749
Little Rock, AR 72205
Office Telephone: 501-526-5935
Email: azhargohar@uams.edu

Sub-Investigators: Jeanne Wei, M.D.
Arny A. Ferrando, Ph.D.
Bryce Marquis, Ph.D.
Sanghee Park, Ph.D.
Nookaew, Intawat, Ph.D.
Eugenia Carvalho, Ph.D.
University of Arkansas for Medical Sciences
4301 West Markham St.
Little Rock, AR 72205

Study location: Donald W. Reynolds Institute on Aging @ UAMS
629 Jack Stephens Drive
Little Rock, AR 72205

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Table of Contents

Table of Events

Event	V.1	V.2	V.3	V.4	V.5	V.6	V.7	V.8	V.9	V.10	V.11	V.12	V.13	V.14
Informed Consent	x													
SLUMS	x													x
Medical History	x													
Meds used	x													
Ht & Wt.	x													
Temp & BP	x													
Blood sample[1]	x													
Ingest D3-cr.		x											x	
Phys. exam[2]	(x)	(x)												
Collect urine for 3 days		x[3]											x[3]	
Fasting before visit		x												x
Blood sample[4]		x												x
Urine sample		x												x
Stool sample[5]		x												x
Strength & functional tests		x						x						x
Practice strength test	x													
SF-36 questionnaire		x						x						x
DEXA scan		x												x
Issue diet records		x						x					x	
Collect diet records			x						x					x
Dispense study product and diary		x	x	x	x	x	x	x	x	x	x	x	x	
Return study product and diary			x	x	x	x	x	x	x	x	x	x	x	x

[1] For criteria-related testing.

[2] Can be done on visit 1 or 2.

[3] Between visits, at home. Calls will be made to remind subjects of the time for collection.

[4] For protocol endpoints.

[5] If subjects unable to provide at this visit, they can take home a collection kit to return at next visit.

1.0 Background and Rationale

Heart failure develops when cardiac muscle becomes weakened and consequently is compromised in its ability to contract, relax, or both. Impaired heart function leads to reduced exercise capacity, which in turn leads to progressive muscle weakness and a vicious cycle of sedentary behavior, weight gain, and subsequent development of metabolic abnormalities and sarcopenia. Approximately 6-10% of individuals over the age of 65 suffer from heart failure (1). The risk of death is 35% in the first year after diagnosis, and decreases to about 10% per year thereafter (2). There are two principal forms of heart failure. Most commonly, heart failure has referred to reduced force of ejection (systolic heart failure). Heart failure may also result from an impaired ability of the heart to relax (diastolic heart failure) (3). Systolic heart failure is often accompanied with diastolic dysfunction (1-2). However, more often the contractile capacity of the heart is not affected, or only slightly impaired, and thus (unlike the case with systolic heart failure) the ejection fraction is usually preserved. This form of heart failure is termed heart failure with preserved ejection fraction (HFPEF), and is the predominant form of heart failure world-wide (4,5). Not only are there different types of heart failure, there is a wide range of potential causes of heart failure, including the natural process of aging (6). Regardless of the specific underlying cause or type of heart failure, there are common pathophysiological responses. Most prominently, impaired exercise capacity, shortness of breath, fatigue and muscle strength are hallmarks of heart failure that lead to decreased physical function, and ultimately sedentary behavior and development of insulin resistance (2). Heart failure is also associated with fluid retention and even edema of the intestinal wall which resultant malabsorption of nutrients (7). Poor health of the intestinal mucosa in heart failure might potentially also influence the gut microbiome. Some long-term consequences of reduced exercise tolerance and malabsorption in long-standing heart failure are loss of muscle mass and the development of cardiac cachexia (7). These responses not only contribute to morbidity and mortality and financial strain on the health care system, but to decreased health-related quality of life.

Treatment of the underlying cause of heart failure would be optimal. Indeed, pharmacological treatment of heart failure predominantly targets the improvement of cardiac performance.

Heart failure may be treated with varying degrees of success with a variety of drugs, including angiotensin-converting (ACE-inhibitors, angiotensin II, beta blockers, diuretics, digoxin, nitrates and others). However, treating systolic heart failure pharmacologically may be quite complex in the elderly. More than 50% of individuals over age 65 with heart failure have at least four co-morbidities (8), and these may complicate therapy of heart failure. Adverse responses to pharmacological therapy are not uncommon, including the fact that both ACE inhibitors and beta blockers can adversely affect muscle function (9). Further, HFPEF is becoming more widely recognized, and to date, despite the expenditure of millions of dollars and years of time and effort, not one large-scale clinical study of a single drug therapy has demonstrated a substantial beneficial outcome. This disappointing reality may reflect the difficulty of treating a syndrome with diverse causes, interacting complex pathogenesis, and multiple co-morbidities with an entirely drug-oriented approach. Reduced exercise capacity is a prominent clinical feature of heart failure. This leads to progressive muscle weakness and a vicious cycle of sedentary behavior, weight gain, and subsequent development of metabolic abnormalities and sarcopenia.

In this study we will perform a randomized clinical trial of a commercially produced nutritional supplement (EAA mixture Essential Blends, Fairbanks, AK) as compared to a placebo in order to determine effects on physical function and health-related quality of life. Subjects will ingest either the EAA mixture product or placebo every day for 12 consecutive weeks. Outcomes will be determined by comparing the results of physical and functional tests from weeks -1 to 6 and 12.

In a pilot study 18 overweight/obese subjects with heart failure (all exceeded 40% body fat) were studied. Nine subjects received 12 weeks of dietary supplementation with 20 g of whey protein consumed daily, while the other nine were controls. Consistent with the acute data showing a limited stimulation of muscle protein synthesis with dietary amino acids in the profile of whey protein in even healthy elderly (i.e., anabolic resistance, Figure 1), supplementation with whey protein failed to improve functional performance. The lack of a demonstrable effect of whey is consistent with the diminished responsiveness to the stimulation of muscle protein synthesis by whey protein in heart failure (10). In contrast, a 2016 UAMS pilot study found that the EAA mixture is better at overcoming anabolic resistance than Ensure Heart Health (see below).

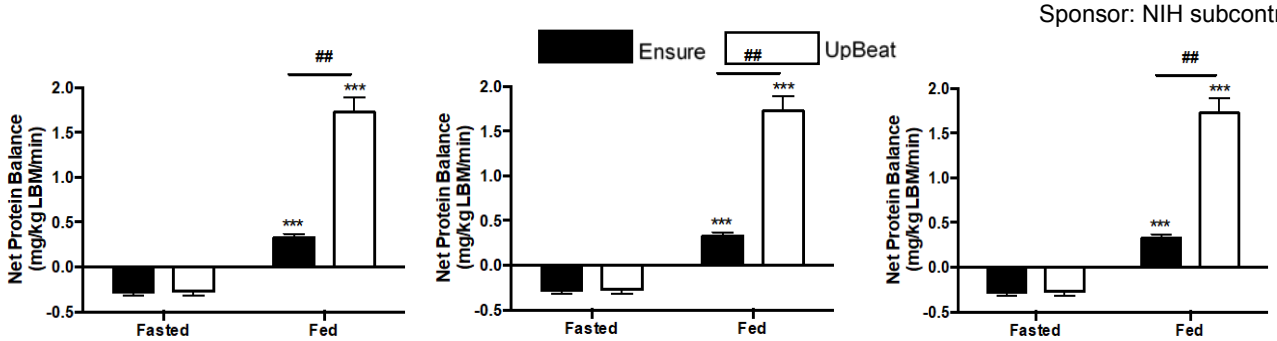

Figure 5. Average rates of Protein synthesis, breakdown and net balance between synthesis and breakdown after consumption of Ensure Heart or UpBeat. **significantly different from fasted state (p<0.005); ***significantly different from fasted state (p<0.0005; ## significantly different between treatments (p<0.005.

2.0 Hypotheses

1. We propose that 12 weeks of regular consumption of EAA mixture will improve physical function in individuals with heart failure as compared to placebo.
2. We propose that regular consumption of EAA mixture will improve health-related quality of life as compared to placebo.

3.0 Study Design and Procedures

This is a randomized, double blind, prospective study examining the effects of 12 weeks' ingestion of either EAA mixture or placebo on physical function. We will also examine several blood parameters and fecal microbiome in both groups of subjects (see section 5.1). Up to 80 subjects ages 60 to 89 will be enrolled to obtain 60 completers (30 per group).

3.1 Study Visits

Visit 1: subjects will come to the UAMS IOA 3rd floor for informed consent discussion. Once consent is obtained, study staff will administer and score the SLUMS test for dementia. If subjects score >20, subsequent study procedures will be performed. A medical history and list of current medications will be obtained (printed from Epic if they are a UAMS patient). Subject height, weight, body temperature and blood pressure will be measured. A blood sample will be drawn (approx. 15 mL) for criteria-related tests if recent results are not available from their medical record. A brief training session will take place where subjects will be introduced to the

isokinetic dynamometer (Cybex) machine and how it is used at the testing visits. They will undergo a practice test on one leg.

A physical exam will be performed at this visit or at visit 2. Based upon the results of the screening blood sample, visit 2 will be scheduled.

Visit 2: Subjects must come to this visit having fasted overnight. They will be asked about any adverse events since last visit. A fasting blood sample (~30 mL) will be drawn to determine baseline values (tests listed below), and a snack will be provided and coffee offered. A urine sample will be collected for dipstick proteinuria. Subjects will be asked to provide a stool sample, but if unable at this visit, they can take home a sample collection kit to use. They will be instructed to collect the stool sample at home on the day of Visit 2 or at UAMS. Subjects will be provided with a drink containing 30mg of a stable isotope of creatine (D-3, Cambridge Isotopes Laboratory, Tewksbury, MA) dissolved in water. They will be asked to collect 3 separate urine samples over the next 72 hours at approximately 24-hour intervals, place them into their freezer, and return them to UAMS at their next visit.

If not performed at visit 1, the physical exam will be performed at this visit. Randomization will be performed as mentioned below. The following assessments will be performed: functional (6-minute walk distance), SF-36 health questionnaire, a whole-body DEXA scan for lean mass measurement, strength (Cybex testing of maximal voluntary contraction of each leg; bilateral hand grip strength). Subjects will be provided with forms and training for recording their dietary intake over a 3-day period during the next week. Study staff will dispense approximately 10 days' worth of study product and a consumption diary for subjects to complete after ingesting each dose. Future visits will be scheduled.

Visits 3-7: Subjects will come to the IOA weekly to return their urine samples, empty supplement containers and consumption diary. They will be asked about any adverse events since last visit. Study staff will dispense more study product and a consumption diary.

Visit 8: Subjects will be asked about any adverse events since last visit. They will return their empty supplement containers and consumption diary. In no particular order, the following assessments will be performed: strength (Cybex testing of maximal voluntary contraction of

each leg; bilateral hand grip strength), functional (6-minute walk distance), SF-36 health questionnaire. Study staff will dispense more study product, a consumption diary, and another 3-day diet diary.

Visits 9-12: Subjects will come to the IOA weekly to return their empty supplement containers and consumption diary. They will be asked about any adverse events since last visit. Study staff will dispense more study product and a consumption diary.

Visit 13: Subjects will come to the IOA to return their empty supplement containers and consumption diary. They will be asked about any adverse events since last visit. Study staff will dispense more study product and a consumption diary. Subjects will be provided with a drink containing 30mg of a stable isotope of creatine (D-3, Cambridge Isotopes Laboratory, Tewksbury, MA). They will be asked to collect 3 separate urine samples over the next 48 hours at approximately 24-hour intervals and return them to UAMS at their next visit. Subjects will be issued a stool sample collection kit to use just prior to visit 14 and another 3-day diet diary.

Visit 14: Subjects must come to this visit having fasted overnight. They will be asked about any adverse events since last visit. They will return their diet records, empty supplement containers, any full supplement containers, their stool sample, and their consumption diary. Study staff will administer and score the SLUMS test for dementia. A fasting blood sample (~30 mL) will be drawn. After the blood sample is drawn, subjects will be offered a meal and beverages. A urine sample will be collected for dipstick proteinuria.
The following assessments will be performed: functional (6-minute walk distance), SF-36 health questionnaire, a whole-body DEXA scan for lean mass measurement, strength (Cybex testing of maximal voluntary contraction of each leg; bilateral hand grip strength). At the end of this visit, subject will have no further study visits.

3.2 Subject Compliance

If a subject consistently reports failing to drink at least 80% of the beverages, or fails to show up for the weekly visit multiple times, they will be dropped from the study. At the weekly visits, study staff will weigh the returning supplement containers and write the mass on the container.

This will allow for calculation of expected decrease in mass over time, which will provide data as to subject compliance.

3.3 Subject Compensation

Subjects will accrue compensation for every visit according to the below table. They will be provided with a check based upon their compensation as of visits 7 and 14. If they were to attend every visit, their total amount would be $300.

Visit	Amount
1	$25
2	$50
3-7 (each)	$10
8	$50
9-12 (each)	$10
13	$35
14	$50

3.4 Randomization

We will use computer randomization to assign subjects to one of two possible treatment groups. The randomization procedure will be stratified by gender. A randomization procedure will be implemented using an Excel spreadsheet. As a participant is identified and enrolled on study, the study coordinator will ascertain gender, which will determine the next randomization.

A: subjects will consume 9g of EAA mixture supplement twice daily for 12 consecutive weeks.

B: subjects will consume 9g of placebo twice daily for 12 consecutive weeks.

3.5 Blinding

This is a double-blinded study. EAA mixture will be provided by Prinova Inc. (Carol Stream, IL). Placebo will be purchased from online or local sources (Pure Protein website, Wal-Mart, etc.). Product containers will be coded so that only the unblinded study staff will know product

identity. After all study visits have been performed and all data entered into a database, the blind will be broken. Although the study products differ in taste, subjects will not be told which products are offered in which flavors.

3.6 Study Products

The active study product is 9g twice a day of EAA mixture, a proprietary formula of Essential Blends, LLC (Fairbanks, AK). Product is a blend of amino acids, flavorings, caffeine and sweeteners (see Appendix) in powder form; packaged in 200g sealed containers. Subjects will dissolve 1 scoop of supplement into ~7 ounces of water and stir/shake until fully dissolved. They will be provided with shaker cups free of charge. Servings will be ingested between meals so that they ingest 2 servings per day. EAA mixture is currently available in only one flavor (lemon/lime).

The placebo product is 9g twice a day of Pure Protein 100% Whey protein (Worldwide Sport Nutritional Supplements, Inc., Bayport, NY). Unblinded study staff will repackage this product into 200g sealed containers which contain the appropriate scoops. See appendix for product information. Subjects will dissolve 1 scoop of supplement into ~7 ounces of water and stir/shake until fully dissolved. Servings will be ingested between meals so that they ingest 2 servings per day.

Subjects will be asked to save empty containers and return them to the study site for accountability purposes. Storage of both products is in a dry area at room temperature. Containers will be labelled identically, with the only difference being the study product code (A or B); the key to which is known only to the unblinded study staff until blinding has been broken at the end of data analysis.

3.7 Sample Storage

Blood and urine samples will be kept frozen at -60 degrees Centigrade or colder once the initial processing has taken place. Stool samples will be kept refrigerated at 4 degrees C until processed. Samples shall be stored in appropriate freezers in the PI's laboratory, located in a restricted area inside the UAMS IOA building. Said freezers are monitored continuously for proper temperature and working condition. Samples will be destroyed only after all data has been analyzed and reported to the sponsor. All blood samples shall be identified using a

unique study acronym. None of a subject's personal identifiers shall be present on any biological sample. Urine samples collected during visits 2 and 14 will be discarded immediately after performing the dipstick test for proteinuria.

4.0 Study Population

Subjects will be recruited using these methods: 1) past subjects that indicated they wanted to be contacted about future studies will be called by study staff to elicit their interest in this study, and 2) the PI will refer potential subjects from her UAMS clinic population to study staff who will then contact the referrals via phone. Once a potential subject has agreed to come to UAMS for an informed consent discussion, an appointment will be made for them to meet with study staff in the research area of the 3rd floor of the Reynolds Institute at UAMS. This study will enroll up to 80 subjects aged 60-89 years inclusive.

4.1 Inclusion Criteria

- Ages 60-89 yrs
- BMI between 18 and 45 kg/m^2
- Any ethnicity
- Presence of mild-to-moderate heart failure (NYHA II or III symptomatology) as evidenced by prescribed diuretics or reported shortness of breath upon exertion.

4.2 Exclusion Criteria

- Allergic to milk or soy products
- Hemoglobin <10 g/dL
- eGFR < 30
- Inability to perform strength and/or functional assessments
- Myocardial infarction in the past 6 months
- Unstable angina
- Moderate-severe heart valve disease
- Infiltrative, restrictive or hypertrophic cardiomyopathy
- Dementia –determined by a SLUMS score of <20

- Currently has inflammatory bowel disease
- Received chemotherapy or radiation therapy within the past 12 months
- Currently undergoing tube feeding
- Currently receiving palliative care for end-of-life circumstance
- Unwilling to refrain from using non-study protein/amino acid supplements during their participation in this study
- If deemed medically unstable by the study physician for any other reason.

5.0 Risks and Benefits

There are no direct benefits for the subjects apart from the possibility getting knowledge of their own results of the assessments and blood tests. Expected risks associated with this protocol are described in detail below. All experimental procedures will be performed by appropriately trained and credentialed personnel.

5.1 Blood, urine and fecal sampling:

The total amount of blood taken will be approximately 100 mL. Subjects will have no noticeable effects. The risks of collecting blood samples include pain, bruising and bleeding.
Urine samples will be tested using commercial dipsticks for proteinuria during visits 2 and 14. Urine collected between visits 1-2 and 13-14 will be analyzed in the PI's lab for the isotope-labelled creatinine to determine skeletal muscle mass (14). There is no risk of collecting voided urine. Fecal samples will be collected at visits 2 and 14. There are no risks associated with collecting fecal samples.

5.2 DEXA scan:

The DEXA scan exposes subjects to approximately ½ of the radiation of one chest x-ray. They will undergo 2 DEXA scans in total.

5.3 Exercise testing (strength and functional):

Subjects may become sore or fatigued during or after the exercise tests. Study staff will remain close to subjects for safety, in the case of dizziness or potential falls. Tests will be paused or stopped if the participant is tired or short of breath. Tests will also be stopped if, for any reason, there is concern about the subject's safety. During the 6-minute walk test, subjects will wear a portable oxygen saturation monitor to allow study staff to observe for desaturation.

5.4 Study supplements:

There are potential allergens in the placebo: milk and soy. All components of EAA mixture (Table 4) are classified as Generally Regarded as Safe (GRAS) by the FDA. They will be provided by PrinovaUSA, which has all necessary certifications for safe production of dietary supplements.

5.5 Confidentiality:

A potential risk to study participants is the potential for loss of confidentiality. Measures to protect the confidentiality of study participants will be implemented as described in the Data Handling and Recordkeeping section below.

5.6 Oral isotope ingestion:

There are no known risks to ingesting 30mg of stable isotope-labelled creatine.

6.0 Data Handling and Recordkeeping

Source documents and CRFs will be stored in a secure area of the PI's laboratory. Access will be limited to study personnel. Documents containing identifiers (except the signed ICF) will be destroyed by shredding approximately 7 years after data analysis is completed or publication of data; whichever is longest. The original, signed ICF will be kept indefinitely. At no time shall Protected Health Information be released to non-study personnel.

The Principal Investigator will carefully monitor study procedures to protect the safety of research subjects, the quality of the data and the integrity of the study. All study subject

material will be assigned a unique identifying code or number. The key to the code (the instrument associating the data with subject identity) will be kept on a password-protected UAMS server, located behind locked doors in a restricted access area of the UAMS campus. Only those individuals listed on the title page of this protocol and their research staff members will have access to the code and information that identifies the subject in this study. This file will be deleted approximately 7 years after data analysis is completed.

7.0 Data Analysis

7.1 Statistical Analysis plan

A repeated measures analysis of covariance (ANCOVA) model will be used to evaluate the effects of EAA mixture and placebo on each parameter. The model will contain EAA mixture versus placebo and time main effects as well as their interaction. Baseline measures will be included in the model as a covariate to control for each subject's starting point. The model will also include factors to account for lean body mass and gender. A non-significant interaction would imply that the EAA mixture or placebo and time effects are not dependent upon one another and that main effects can be tested. The significance of the interaction and main effects will be determined using a 5% α-level. Covariates that may need to be accounted for such as age and lean body mass may be added to the model as a separate exploratory analysis.

7.2 Significant Interaction Analysis Plan

A significant interaction suggests that the EAA mixture or placebo effect is dependent upon the time effect. This implies the EAA mixture or placebo effect will have to be assessed separately within each time point, and vice versa. Since the two EAA mixture and placebo groups are independent, an analysis of covariance (ANCOVA) model will be used to assess the EAA mixture or placebo effect for each time point (midpoint & final). A repeated measures analysis of variance (ANOVA) model will be used to assess the time effect for EAA mixture or placebo, where the repeated measure is time. A Bonferroni-corrected α-level of 2.5% will be used to determine the statistical significance of these tests.

7.3 Sample Size Calculation & Power Analysis

A total of 60 completed subjects will be required for this study with 30 subjects per EAA mixture and placebo group. The following power calculations are based on the analysis of the results of the six minute walk, leg strength, grip strength and 10 meter gait speed from our pilot studies. Insulin sensitivity and plasma lipids will be secondary end points. These calculations assume the "worst case" scenario where a significant EAA mixture or placebo-by-time interaction is detected and the covariates are not significant. In this case, the EAA mixture or placebo effect would be assessed separately within each time point, and the time effect would be assessed for each group.

7.4 EAA mixture or placebo Effect

The following power calculations are based on a two-sample t-test which is being used as an approximation of the ANCOVA model. With 30 subjects in each EAA mixture and placebo group and an α-level of 2.5%, a two-sample t-test will have approximately 80% power to detect effect sizes of 0.82 standard deviation units or larger. **Table 4** presents the hypothesized group means for each endpoint as well as the corresponding EAA mixture or placebo effect and power estimate. These estimates correspond to approximately 25% (6-minute walk) to 50% (gait speed) of the differences in the values between individuals with heart failure vs age-matched healthy controls as determined in our Phase I protocol. Our previous outcome studies with EAAs indicate that these levels of improvement are expected (e.g., 11, 12, 13).

Table 4. Hypothesized group means and within group standard deviation used to estimate the power of the nutritional therapy effect when N=60 (30 per group) are enrolled. The calculations assume that an α-level of 2.5% will be used to determine statistical significance.

Endpoint	Hypothesized Mean Changes[#]		σ_{Δ}[$]	Effect Size	Power (%)
	Control	Nutritional product			
6 Minute Walk[a]	0	105.1	89.3	1.18	98.67
Leg Strength[a]	0	11.0	13.3	0.83	81.31
Grip Strength[a]	0	4.1	4.6	0.89	87.19
10m Gait Speed[a]	0	0.23	0.28	0.82	80.80

[#]Means of the baseline and post-treatment changes for each group.
[$]Estimated within group standard deviation of the changes.
[a]Estimates of σ_Δ were obtained from pilot studies performed at the UAMS Pepper Center.

7.5 Time Effect

The following power calculations are based on a paired t-test which is being used as an approximation of the repeated measures ANOVA model. With 60 subjects within each time point, a paired t-test using a 2.5% α-level will have 80% power to detect effect sizes of 0.41 standard deviations or larger. Note that the variance of the difference (σ_Δ^2) in the measures can be calculated using the following formula: $\sigma_\Delta^2 = \sigma_M^2 + \sigma_F^2 - 2\rho\sigma_M\sigma_F$, where ρ is the correlation between the mid-point (8 weeks) and final (16 weeks) measures, and σ_M and σ_F represent the standard deviations for the mid-point and final assessments, respectively.

Assuming the standard deviations for the mid-point and final measures are similar, the equation can be simplified to $\sigma_\Delta^2 = 2\sigma_\Delta^2(1-\rho)$. Using the simplified formula and our estimates of the standard deviations from Table 4, **Table 5** presents the detectable differences that correspond to an effect size of 0.41.

Table 5. Detectable differences associated with an effect size of 0.41.

Endpoint	ρ	σ_Δ	Detectable Difference
6 Minute Walk	0.25	109.4	44.9
	0.50	89.3	36.6
	0.75	63.1	25.9
Leg Strength	0.25	16.3	6.7
	0.50	13.3	5.5
	0.75	9.4	3.9
Grip Strength	0.25	5.6	2.3
	0.50	4.6	1.9
	0.75	3.3	1.4
Gait Speed	0.25	0.34	0.14
	0.50	0.28	0.12
	0.75	0.20	0.08

7.6 Urine analyses

Urine samples will be analyzed for the presence of D3-creatinine (mass spectroscopy in PI's lab) and unlabeled creatinine (Labcorp, Dallas, TX). Subjects will be sent home with 3 labelled urine collection containers to use at appropriate intervals after ingestion.

7.7 Fecal analyses

A subset of 20 subjects (10 from each group) will be asked to provide a fecal sample using a standard stool collection kit (DNA/RNA Shield™ Fecal Collection Tube from Zymo Research).

Any participants who prefer to take the collection kit home will be provided a shipping container and explicit instructions on sample collection, storage and shipping. The stool collection kits contain a nucleic acid stabilizer solution that allows room temperature storage and shipment of collected samples. Once acquired by the laboratory, samples will be kept refrigerated at 4 degrees C until processed, within 20 days. Fecal analysis will analyze the microbiome diversity in the subjects. Bacterial species (eg. bacteroidaceae, pseudomononadaceae, ruminococcocacease) will be identified by sequencing the bacterial DNA. These results will be reported back to the investigators for analysis of microbiome gut diversity between heart failure patients on control supplement versus EAA supplement. Results will be stored for studies. The microbial DNA will be enriched and purified at a lab on the UAMS campus using a modification of the standard Zymobiomics DNA purification kit (Zymo Research) to obtain high quality and high molecular weight DNA.

7.8 Blood analyses

In addition to the blood testing for eligibility, blood samples will be collected solely for the purpose of experimentation. The blood will be used to measure brain naturetic peptide, creatinine, complete blood count (CBC), glucose, insulin, plasma lipids, profile of cardiac aging proteins including serum response factor, hemoglobin A1c, plasma acylcarnitines, mitochondrial function, and possibly amino acid concentrations.

8.0 Ethical Considerations

This study will be conducted in accordance with all applicable government regulations and University of Arkansas for Medical Sciences research policies and procedures. This protocol and any amendments will be submitted and approved by the UAMS Institutional Review Board (IRB) to conduct the study.

The formal consent of each subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. All subjects for this study will be provided a consent form describing this study and providing sufficient information in language suitable for subjects to make an informed decision about their participation in this study. The

person obtaining consent will thoroughly explain each element of the document and outline the risks and benefits, alternate treatment(s), and requirements of the study. The consent process will take place in a quiet and private room, and subjects may take as much time as needed to make a decision about their participation. Participation privacy will be maintained and questions regarding participation will be answered. No coercion or undue influence will be used in the consent process. This consent form must be signed by the subject and the individual obtaining the consent. A copy of the signed consent will be given to the participant, and the informed consent process will be documented in each subject's research record.

9.0 Dissemination of Data

Results of this study may be used for presentations, posters, or publications. The publications will not contain any identifiable information that could be linked to a participant.

10.0 References

1. McMurray FF, Pfeffer R. Heart failure. Lancet 2005; 365:1877-89.
2. Chronic Heart Failure: National clinical guidelines for diagnosis and management in primary and secondary care: partial update [Internet]. National Clinical Guideline Centre (UK). London: Royal College of Physicians (UK) 2010 Aug; PMID:22741186
3. Ferrari R, Bohm M, Cleland JG, Paulus WJ, Pieske B, Rapezzi C, Tavazzi L. Heart failure with preserved ejection fraction: uncertainties and dilemmas. Eur J Heart Fail 2015; 17:665-71.
4. Owan TE and Redfield MM. Epidemiology of Diastolic Heart Failure. Progress in Cardiovascular Disease 2005; 47:320-332.
5. Bhatia RS, Tu JV, Lee DS, Austin PC, Fang J, Haouzi A, Gong Y, Liu PP. Outcome of heart failure with preserved ejection fraction in a population-based study. N Engl J Med 2006 Jul; 355(3):260-9.
6. Azhar G, Wei JY. The demographics of gaining and its impact on the cardiovascular health. Curr Cardiovasc Risk Report 2015; 4:1-6.
7. Azhar G, Wei J. Cardiac cachexia. Currr Opin in Clin Nutr and Met Care 2005; 9:18-23.
8. Braunstein JB, Anderson GF, Gerstenblith G, Weller W, Niefeld M, Herbert R, Wu AW. Noncardiac comorbidity increases preventable hospitalizations and mortality among Medicare beneficiaries with chronic heart failure. J Am Coll Cardiol 2003 Oct 1; 42(7):1226-33. PMID:14522486
9. Bacurau AV, Cunha TF, Souza RW, Voltarelli VA, Gabriel-Costa D, Brum PC. Aerobic exercise and pharmacological therapies for skeletal myopathy in heart failure: Similarities and differences. Oxid Med Cell Longev 2016; 437-467. PMID:26904163.

10. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. Am J Clin Nutr 2005 Nov; 82(5):1065-1073.
11. Ferrando AA, Paddon-Jones D, Hays NP, Kortebein P, Ronsen O, Williams RH, McComb A, Symons TB, Wolfe RR, Evans W. EAA supplementation to increase nitrogen intake improves muscle function during bed rest in the elderly. Clin Nutr 2010 Feb; 29:18-23.
12. Borsheim E, Bui QU, Tissier S, Kobayashi H, Ferrando AA, Wolfe RR. Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. Clin Nutr 2008 Apr; 27(2):189-195. PMCID:PMC2430042
13. Ferrando AA, Bamman MM, Schutzler SE, Spencer HJ, Evans RP, Wolfe RR, Increased nitrogen intake following hip arthroplasty expedites muscle strength recovery. J Aging: Research and Clinical Practice 2013; 2(4):369.
14. Richard V. Clark, Ann C. Walker, Robin L. O'Connor-Semmes, Michael S. Leonard, Ram R. Miller, Stephen A. Stimpson, Scott M. Turner, Eric Ravussin, William T. Cefalu, Marc K. Hellerstein, and William J. Evans. Total body skeletal muscle mass: estimation by creatine (methyl-d) dilution in humans. J Appl Physiol (1985). 2014 Jun 15; 116(12): 1605–1613. Published online 2014 Apr 24. doi: 10.1152/japplphysiol.00045.2014

11.0 Appendices

11.1 Study Product Information

EAA mixture

His	3.5%
Ile	7.1%
Leu	14.3%
Lys	6.0%
Met	3.3%
Phe	8.8%
Thr	5.3%
Trp	1.4%
Val	9.25%
Glutamine	1.2%
Carnitine	2.9%
Nicotinamide	1.5%
Vitamin C	1.5%
Caffeine	0.6%
Sucralose and other flavorings and preservatives	28.3%

Placebo: Pure Protein – Vanilla Creme flavor

Directions: *For adults, take one (1) scoop (39 g), one to two times daily. For maximum muscle support consume 1-2 scoops twice per day, with at least 1-2 scoops taken immediately after exercise.*

Nutrition Facts

Serving Size 1 Scoop (39g)
Servings Per Container about 23

Amount Per Serving	
Calories 150	Calories from Fat 25
	%Daily Value*
Total Fat 2.5g	**4%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 80mg	**27%**
Sodium 100mg	**4%**
Potassium 160mg	**5%**
Total Carbohydrate 8g	**3%**
Dietary Fiber 0g	**0%**
Sugars 2g	
Protein 25g	**50%**
Vitamin A 0%	Vitamin C 0%
Calcium 20%	Iron 0%
Phosphorus 10%	Magnesium 4%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Potassium		3,500mg	3,500mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g
Protein		50g	65g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

INGREDIENTS: Protein Blend (Ultrafiltered Whey Protein Concentrate [which contains Beta-lactoglobulin, Alpha-lactalbumin and Glycomacropeptides], Microfiltered Whey Protein Isolate), Maltodextrin, Natural and Artificial Flavors, Cellulose Gum, Soy Lecithin, Xanthan Gum, Dicalcium Phosphate, Calcium Carbonate, Acesulfame Potassium, Sucralose.

Contains milk and soy ingredients.

Typical Amino Acid Profile (milligrams per 39 g scoop)**

Essential Amino Acids		Nonessential Amino Acids	
Histidine	490 mg	Alanine	1,139 mg
Isoleucine	1,460 mg	Arginine	640 mg
Leucine	2,531 mg	Aspartic Acid	2,980 mg
Lysine	2,127 mg	Cysteine	601 mg
Methionine	562 mg	Glutamic Acid	3,919 mg
Phenylalanine	808 mg	Glycine	444 mg
Threonine	1,783 mg	Proline	1,415 mg
Tryptophan***	485 mg	Serine	1,299 mg
Valine	1,376 mg	Tyrosine	728 mg

***L-Tryptophan is naturally occurring, not added.
**approximate values

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