Protocol Title	A physical activity program in end-stage liver disease: pilot study assessing changes in physical fitness, sarcopenia, and the metabolic profile
Principal Investigator	**Arny Ferrando, PhD**[1]
Co-Investigator(s)	Andres Duarte-Rojo, MD, MSc, DSc[2] Thaddeus Bartter, MD[3] Aldo Montano-Loza, MD, MSc, PhD[4]

[1]Center for Translational Research in Aging and Longevity

[2]Division of Gastroenterology and Hepatology

[3]Division of Pulmonary and Critical Care Medicine

University of Arkansas Medical Sciences
4300 W. Markham
Little Rock, AR 72032

[4]Division of Gastroenterology

University of Alberta
1-20A Zeidler Ledcor
Edmonton, Alberta, Canada

Location(s) of Study	University of Arkansas for Medical Sciences University of Alberta
Version	**10**
Date	10/10/2018

ABSTRACT

Patients with end-stage liver disease (ESLD) have poor physical fitness and muscle wasting (sarcopenia). Both conditions have been associated with elevated mortality before and after liver transplantation (LT). Limited data have shown that exercise benefits patients with chronic liver disease/cirrhosis, however, there are no studies with ESLD patients potentially eligible for LT. Effectively improving physical fitness and sarcopenia in ESLD should positively impact clinical outcomes before/after LT. The primary aim of this study is to improve both physical fitness and sarcopenia in ESLD patients through a 12-week physical training program. Secondary aims will focus on changes in anthropometrics, body composition, quality of life, and metabolic profile. This is a randomized clinical trial including 50 patients, with half allocated to the active group (physical training program) and half to standard of care. Physical training program will consist on achieving ≥10,000 steps/day with an increase of ≥4000 above the baseline, facilitated by the feedback from daily step counts and behavioral modification therapy (favoring change towards a more active lifestyle). All participants will have to wear a pedometer bracelet (Fitbit) throughout duration of study (reads blinded to controls), and will receive a daily essential amino acid (EAA) supplement containing approximately 2.25 g of L-leucine along with nutritional consultation. Cardiorespiratory exercise test (CRET), 6-minute walk test (6MWT), and abdominal CT scan will be performed in all participants before and after the period of study to assess for physical fitness and sarcopenia. Anthropometrics, bioelectrical impedance, whole-body DEXA scan, sickness impact profile questionnaire, and the metabolic profile will be obtained at baseline and after completion of the study as well. Blood EAA levels, myostatin, and insulin-related growth factor-1 will be determined at baseline, and at week 12 of study. Durability of changes 6 months post-LT will be assessed as well, when applicable.

INTRODUCTION

End-stage liver disease (ESLD) is the final consequence of cirrhosis and portal hypertension, causing great morbidity and mortality, and necessitating liver transplantation (LT) to preserve life.(1) Physical fitness is defined as the ability to carry out daily tasks with vigor and alertness, without undue fatigue.(2) Compared to healthy peers, patients with ESLD have a poor physical fitness, and deterioration progresses along with liver failure. Patients with chronic liver disease (with or without cirrhosis) have low to limited activity, with daily pedometer counts between 5000-7000 steps per day, what is way below a minimum recommended of 10,000 daily steps, for a "somewhat active" individual.(3-5) There are several reasons for patients with cirrhosis having low to limited physical activity. Some of the most common contributing factors include chronic fatigue, muscle wasting, obesity, intentional inactivity (either physician-prescribed or self-imposed due to disease misconceptions), poor motor and visuospatial coordination in relation to hepatic encephalopathy (HE), covert HE-associated fear of falling, ascites and fluid overload limiting mobility, and concomitant cardiopulmonary diseases (hepatopulmonary syndrome, portopulmonary hypertension, cirrhotic cardiomyopathy).(6, 7)

Severe skeletal muscle loss or sarcopenia is one of the end results of a limited physical fitness, and it is also a major feature of malnutrition. Notably, sarcopenia and HE are closely related and they can perpetuate or aggravate each other. Since skeletal muscle represents the major organ participating in extrahepatic ammonia metabolism, it is not surprising to find that patients with sarcopenia and malnutrition have a higher risk for HE.(8, 9) On the other hand, HE negatively impacts muscle mass by affecting motor coordination and physical activity, and experimental data have also shown that hyperammonemia has a detrimental impact on skeletal muscle by favoring cell autophagy.(10)

Physical fitness and sarcopenia are both common conditions affecting 41-88% of patients with ESLD in the LT waitlist,(11, 12) and they have been associated with poor clinical outcomes. Both physical fitness and sarcopenia have been independently associated with poor survival before and after liver transplantation.(11-16) Sarcopenia has also been linked to a longer hospital stay during the LT admission, and with a higher risk for peri-operative infectious complications. A recent study has noted that addition of sarcopenia to MELD (model for ESLD) is a better predictor of waitlist mortality and dropout than MELD by itself.(17) Due to its paramount importance in predicting LT outcomes, two groups have independently proposed the addition of a sarcopenia measure to MELD (MELD-psoas and MELD-sarcopenia) in order to have a more accurate prognostication of mortality (pre- and posttransplant), and to better prioritize patients for LT.(17, 18) In spite of all the above evidence, physical fitness and sarcopenia have both been frequently overlooked as predictive factors for adverse outcomes in LT.

Cardiorespiratory endurance is a measurement of physical fitness obtained through cardiorespiratory stress tests. Two common markers of cardiorespiratory endurance are the VO_{2max} obtained by cardiorespiratory exercise testing (CRET), and the distance strolled in the 6-minute walk test (6MWT). The VO_{2max} is the point at which, during a CRET, the consumption of oxygen cannot be augmented in spite of increased exercise intensity (Figure 1a). After the VO_{2max} is reached, there is a disproportionate increase in VCO_2 production with respect to VO_2 consumption, which is known as the anaerobic threshold (Figure 1b). The anaerobic threshold corresponds to the point at which lactic acid rises systemically during incremental exercise, as a result of anaerobic glycolysis.

In spite of changes in physical fitness and sarcopenia being attributed to a clinical condition (*i.e.* ESLD) evidence points to lifestyle changes as a major player in its causation and perpetuation. A

provocative study has shown that LT by itself is not capable of improving cardiorespiratory endurance, as shown in the lack of significant improvement in VO_{2max} in 20 patients who had it tested before and more than 1 year after LT. The mild increase in VO_{2max} from 63% to 71% - and 25% of patients showing decreased VO_{2max} - mostly correlated with the improvement in hemoglobin, and stopping of the beta-blocker. Although there was likely some improvement in skeletal muscle physiology, as suggested by changes in peak lactate concentration, LT had no major impact in aerobic capacity and cardiorespiratory endurance.(19) This suggests that, even though poor physical fitness and sarcopenia are primarily driven by ESLD, restoration of normal liver function by means of LT is not enough to positively impact physical fitness. Likely in relation to the lack of changes in lifestyle favoring healthy habits, patients progressively gain weight after liver transplantation,(20) and it is not surprising to find a high prevalence of the metabolic syndrome (>50% after the first year post-LT; >50% of what is observed in the general population), particularly hypertension and diabetes mellitus (>60% after first year post-LT), and are at great risk for cardiovascular complications.(21)

Although there are some conflicting data regarding what benefits physical activity or exercise confers to various morbid states, it is in general conceived that improving physical fitness is beneficial, both by preventing deterioration of a state of health, and by helping diseased conditions through adaptation.(22, 23) In chronic liver disease, and particularly in the setting of ESLD, there is a great paucity of data regarding the beneficial effects that exercise or a physical activity program can offer to patients. Actually, to date, there is no robust evidence from large randomized trials to sustain that the deterioration in physical fitness and sarcopenia that patients with ESLD are exposed to, is reversible by any type of intervention.

Pattullo *et al* included 16 patients with chronic hepatitis C - 6 of whom were cirrhotic - in a 24-week exercise and dietary program. Exercise consisted on walking and patients were encouraged to increase their daily step count using a pedometer to self-monitor their progress. With the aid of behavior modification therapy we were able to increase daily step counts from a baseline of 6853 (2440-9533) to 10967 (7959-13566) steps/day, and there were significant improvements in fasting glucose, insulin resistance (HOMA-IR (3.6 [2.8-4.9] to 2.1 [1.8-3.6]), BMI (36 [31-38] to 31 [29-36]), waist circumference, fatigue and mood scores. These changes were paralleled by significant drops in ALT, AST, and GGT levels, and by increased adiponectin, and decreased leptin. Calorie restriction had a median of 33% during the intervention.(5) In a total of 8 patients with cirrhosis initially included in this study, we could observe an improvement in quality of life as well, reflected by the physical component summary of SF36 (specifically on physical function, bodily pain, and vitality).(24) With a similar design, Konishi *et al*, were able to demonstrate that in 16 patients with chronic hepatitis C, an increase in daily step counts from around 5500 to over 7000 during a period of 6 months had a positive impact in insulin resistance (HOMA-IR), BMI, waist circumference, ALT, and leptin levels. Of note, this study provided standardized nutritional counseling, but no changes in caloric intake were noted.(4) In a randomized clinical trial, Soriano *et al* showed that a period of 12 weeks of supervised moderate exercise thrice-weekly plus 10 g/d of oral leucine (n=8) caused an increase in albumin levels, muscle mass (as reflected by the thigh circumference), cardiorespiratory endurance (6MWT increased from 321±36 to 417±33 m), and quality of life (according to SF36). None of these changes were noted in the control group (n=9).(25) We recently completed a randomized clinical trial consisting of 12 weeks of a supervised physical training program in patients with cirrhosis. Out of 23 patients included, 11 were allocated to moderate exercise/kinesiotherapy for about 70 minutes per session, three times per week, at a cardiac rehabilitation center. Remaining patients continued with standard of care. Phase angle (PhA) from bioelectrical impedance was used to evaluate muscle mass. We showed a trend for improved muscle mass in the exercise group, as PhA improved in 63% of patients compared to 25% of the control group (p=0.10).

Remarkably, branch-chained fatty acids (particularly L-leucine) administration has been recently associated with decreased skeletal muscle autophagy and reversal of protein-breakdown signaling. Myostatin is one of the mail players driving autophagy and blocking protein synthesis through inhibition of the mammalian target of rapamycin, and it has been found to be elevated in patients with cirrhosis. It is thought that hyperammonemia increases myostatin, then facilitating to impaired proteostasis with the end result of muscle loss or sarcopenia.(26) Finally, insulin-like growth factor 1 (IGF-1) is a trophic factor directly affecting muscle growth and repair, and it is known to be induced with exercise and L-leucine supplementation (Church. J Am Coll Nutr 2016;in press).

Although physical fitness is a modifiable risk factor, there are no studies to date assessing whether an exercise program aiming to improve cardiorespiratory endurance and physical fitness while on the waiting list could potentially improve posttransplant outcomes. One of the concerns with an exercise program in patients with cirrhosis and ESLD is its safety. Two of the perils that could possibly occur as a consequence of exercise are episodes of gastrointestinal (variceal) bleeding and/or HE. Although there are pathophysiologic and anecdotic evidence supporting that an increased risk for these complications is plausible, there are no clinical data to substantiate the concept. At least 9 published studies(4, 5, 11, 25, 27-30) have subjected patients with cirrhosis (± ESLD) to physical activity programs or exercise stress testing - intensity varying from a daily low intensity (estimated VO_{2max} of 35-50%) to a single bout of moderate-to-high intensity exercise (VO_{2max} 60-100%) - with no documentation of adverse reactions. Particularly no episodes of variceal bleeding or HE were observed. However, it is unknown whether such complications were intentionally investigated in any of the above-mentioned studies, as none of them was designed to identify and correlate such complications of ESLD to physical activity.

In an interesting study, Garcia-Pagan *et al* submitted patients with cirrhosis to a low-to-moderate exercise regimen while measuring the hepatic venous pressure gradient (HVPG) and were able to find out that exercise increases the HVPG by 21% at a 50% maximum work load. This was followed by a return to normal after 5 minutes of stopping physical activity.(31) In a subsequent study from the same group, Bandi *et al* showed that the increase in HVPG during exercise could be abolished if the patient was given a beta-blocker before the exercise bout.(32) Since the HVPG is the best available marker to determine portal hypertension and the risk for variceal bleeding, data above suggest that the risk of variceal bleeding acutely increases during exercise, whenever the patient is not receiving a beta-clocker. However, our randomized clinical trial was able to show a decrease in the HVPG by the end of the study period in the active group contrasting the expected increase observed in controls (HVPG: -2.5 [-5.25 to 2] *vs* 4 [0.25 to 8], respectively, p=0.007).(33) This surprising finding brings to light that there might be some other benefits from physical fitness that we are yet unable to see. Moreover, no cases of variceal bleeding were observed in this and other clinical trials evaluating the effect of an exercise program in cirrhosis.(5, 25, 33)

Ammonia, a byproduct of nitrogen metabolism, is the most important factor associated with HE.(34) During exercise, and mostly under anaerobic conditions, breaking of ATP will result in net production of ammonia, what could expose patients with ESLD to hyperammonemia and episodes of HE. In sports medicine, the ammonia threshold, which corresponds to the deflection point at which ammonia production increases disproportionately during CRET (Figure 1c), has been used as a marker of exercise endurance. Actually, the increase in blood ammonia during exercise is directly related to the VO_{2max} such that a more efficient aerobic capacity is associated with higher ammonia thresholds (less ammonia production for same VO_{2max}). Although the ammonia threshold grossly coincides with the inflection point defining the change from aerobic to

anaerobic metabolism (anaerobic and lactate threshold)(35, 36) there are no data in patients with ESLD. Given that ammonia uptake is severely reduced in ESLD, it is possible for patients with ESLD to be exposed to hyperammonemia under conditions of aerobic exercise, such that exercise could become a hazard even before reaching strenuous levels or exhaustion. Although not systematically investigated, there have been no cases of HE reported in clinical trials evaluating the effect of exercise in cirrhosis.(5, 25, 33)

Every year about 6000 LT are performed in the US, and currently, there is a growing interest to explore the beneficial impact exercise can provide to patients with cirrhosis and ESLD through a structured physical training program. As based on data above, such a program may improve cardiorespiratory fitness and endurance, obesity and its associated metabolic complications (including less insulin resistance), sarcopenia, biomarkers of liver function, quality of life, and even portal hypertension. For hepatologists working on liver transplantation this may translate into reduced dropout from the transplant list, reduced length of hospitalization after transplant, better clinical outcomes including survival, and quality of life,(11, 13, 37) (12, 14) Furthermore, the incidence of obesity, metabolic syndrome, and cardiovascular complications after liver transplantation could be halted by training patients to change their lifestyle into a more physically active one.

We currently spend great resources into avoiding substance abuse relapse and improving patient's compliance during the LT evaluation, but have omitted education regarding healthy lifestyle habits including physical activity and exercise, what might bring enormous benefits to LT worldwide. For patients with cirrhosis and ESLD, the period before liver transplantation is full of self-reflection and insight, education, and lifestyle changes. These behavioral tuning has allowed keeping the rate of alcohol recidivism at no more than 10% in most transplant centers, and a low prevalence for the use of tobacco products after LT. It is expected that any recommendation or prescription regarding physical activity would have greater acceptance and a more durable effect if properly administered once patients have reached ESLD, or while they are being evaluated for LT, as they become very receptive to make changes in their lifestyle as far as it helps to facilitate transplant candidacy. However, unlike diabetes mellitus and cardiac diseases, it is unknown for ESLD patients what the mode, intensity, and duration of exercise that will provide improved outcomes are. More importantly, there is a lack of knowledge regarding the safety of exercise with respect to hyperammonemia and occurrence of HE.

SPECIFIC AIMS

The primary aims of current study are to:

- Improve the physical fitness of patients with ESLD whom are potentially eligible for liver transplantation with a 12-week physical training program.
    - The hypothesis is that only patients enrolled in the active arm will improve their physical fitness, as determined by cardiorespiratory stress test and 6-minute walk test.
- Improve sarcopenia in patients with ESLD whom are potentially eligible for liver transplantation with a 12-week physical training program
    - The hypothesis is that only patients enrolled in the active arm will show an increase in the skeletal muscle area, as determined by CT-scan.

As secondary aims, this 12-week training program will look into:

- The changes in the ammonia and anaerobic/lactate thresholds at the end of the study for each group.
    - It is expected to find increased ammonia and anaerobic/lactate thresholds in the active arm, when compared to the control arm.
- The changes in anthropometrics (weight, BMI, waist circumference), and body composition at the end of the study for each group.
    - It is expected to find improved anthropometrics, decreased fatty mass, and increased fat-free mass in the active arm, when compared to the control arm.
- The changes in quality of life at the end of the study for each group.
    - It is expected to find improvements in quality of life in the active arm, when compared to the control arm.
- Safety of the physical training program, by systematically documenting episodes of variceal bleeding, and hepatic encephalopathy.
    - No increased risk for complications such as GI bleeding and encephalopathy is expected for the active arm with current research design.
- The changes in the metabolic profile (fasting glucose, fasting insulin, lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides) at the end of the study for each group.
    - It is expected to have an improved metabolic profile in the active arm, when compared to the control arm.
- The changes in myostatin and insulin-related growth factor 1 (IGF-1) before and after 12 weeks of study.
- In patients undergoing liver transplantation, extension of training program benefits 6 months after liver transplant (reassessment of physical fitness, sarcopenia, anthropometrics, body composition, and metabolic profile).
    - Durability of the beneficial benefits of the training program will be expected across all evaluated variables, with no changes in the control arm after transplant.

RESEARCH STRATEGY

Significance

Unlike other chronic diseases (*i.e.* diabetes mellitus), there are no recommendations for physical training and exercise in patients with cirrhosis. This derives from the fact that there is a paucity of data on the physiologic effects of exercise in patients with cirrhosis and portal hypertension, as well as on the benefits that such training can bring in the to long term. Moreover, some safety concerns have been raised in the past particularly with regards to an increased risk for variceal bleeding and HE. Apart from a few pilot studies using supervised exercise as an intervention there are no clinical trials addressing the benefits of an exercise program in patients with ESLD. Furthermore, no study has looked into how a training program can translate into benefits after LT, and since LT patients have a very high risk for metabolic and cardiovascular disease, such a study is highly awaited.

Innovation

Of the few studies on physical training in cirrhosis there is not a single one balancing the intervention to be effective, reproducible, feasible, and widely available. Moreover, safety has not been systematically investigated. Although supervised exercise at a physical rehabilitation center is effective and reproducible, it is not possible for most patients to join such a program, and these are definitively not widely available. Prior studies using a waist pedometer - a feasible and available design - might have several flaws: failure of the equipment, incapacity to measure all daily activities, lack of knowledge on cardiorespiratory endurance to tailor physical efforts, and no objective measure to assess efficacy of the intervention. Thus, both efficacy and reproducibility are affected.

Current study has a balanced approach: duration and intensity of physical training have been adjusted to provide positive results as based on prior experience (both from supervised and pedometer-based studies), there are objective and accurate evaluations of cardiorespiratory endurance before and after intervention allowing to safely tailor the intensity of physical activities, behavioral modification therapy is included to facilitate changes in lifestyle, relevant safety concerns have been addressed and data will be collected to facilitate further safety refinement, the equipment to be used (bracelet pedometer) has the highest quality and reliability while allowing to correlate effort to changes in heart rate (such as in a supervised exercise program), and we are innovating with the use of PhA and bioimpedance vector analysis to reliably assess changes in body composition. Moreover, in patients undergoing transplantation, post-LT determinations have been considered to investigate duration of the effect in a population that has a very high mortality. Results from present study would provide physiological basis and preliminary results on efficacy and safety in order to develop a multicenter clinical trial aiming to show improved clinical outcomes (survival and dropout from waiting list before LT; survival, quality of life, metabolic and cardiovascular complications after LT) in patients with ESLD, as well as a future policy for exercise training in patients with ESLD.

APPROACH

All patients referred for an evaluation for liver transplantation, as well as patients with ESLD from hepatology clinics at UAMS, will be screened for study and invited to participate, when applicable. A total of 50 patients will be included in study. Inclusion and exclusion criteria will be as follows:

Inclusion criteria

1. Age 40 to 70.
2. Cirrhosis, any cause, defined as:
    a. Biopsy-proven.
    b. Two or more of the following: albumin <3.5 g/dL, INR >1.3, radiologic or endoscopic evidence of portal hypertension.
3. Creatinine <2.0 mg/dL.
4. Physiologic MELD ≥10.
5. Decompensated cirrhosis with active or history of variceal bleeding, ascites, hepatic encephalopathy, or jaundice.
6. Potential transplant candidate as per UAMS criteria.

Exclusion criteria

1. Large gastric or esophageal varices with contraindication to use beta-blockers.
2. Persistent hepatic encephalopathy grades 2-4.
3. Prior diagnosis of hepatocellular carcinoma, or hepatic hydrothorax (with prior repeated thoracocentesis).
4. Cirrhotic cardiomyopathy or congestive heart failure, pulmonary vascular complications, known active coronary artery disease, syncope, and cardiac dysrhythmias.
5. Physical impediment to perform a cardiorespiratory fitness test.
6. Use of implantable defibrillator or a pacemaker.

Note: all inclusion / exclusion criteria will be obtained from routine investigations available during transplant evaluation, as part of the standard of care.

Description of Study Interventions

Physical Activity

After signing of informed consent patients will be given a Fitbit to measure all physical activity throughout the day. The Fitbit is a digital pedometer inside of a bracelet capable of continuously measuring all physical activities (24/7) and heart rate. Each subject should wear her/his bracelet during the whole duration of the study, for a total of 14 weeks. There will be a 1-week delay between enrollment into the study and the initial study's appointment, in order to obtain the baseline physical activity from each subject (corresponds to week -2, see Study Calendar). Physical activity and heart rate logs will be electronically downloaded to the investigator's computer during each follow up visit.

Physical activity prescription will target ≥10,000 steps/day, taking into account all activities performed throughout the day, although the increment in activity should be no less than 4000 steps/day above the baseline. Apart from walking, there will be no physical activity that would be favored in particular to all participants, but each one will be able to choose and perform the type of exercise of their preference. The VO_{2max}, ammonia, lactate and anaerobic thresholds observed on each subject will help better determine the intensity of exercise each patient should be able to tolerate safely.

Nutritional Intervention

Dietary advice will be provided at the beginning of the study and individually tailored to the participant's usual eating habits, with the following modifications:

- High in low glycemic-index foods and fiber
- Low in fat and cholesterol
- 500 kcal/d calorie restriction if obese
- A light snack at night.
- Protein amount at 1.2-1.5 g/kg/d
- Low sodium (<2000 mg/d) and water restriction to be defined as per standard of care.

Moreover, patients will receive a 10 g bid essential amino acid (EAA) supplement containing approximately 2.25 g/d of L-leucine and 5.2 g of branch-chained amino acids (Reginator, Prinova, Carol Stream, Illinois). This supplement should not affect the total daily caloric intake, or sodium restriction. The intention is to favor healthy food eating habits, along with enough protein to support muscle repair and building. Compliance to the dietary behavior changes will be assessed every 2 to 4 weeks by the LT nutritionist.

Behavioral Modification
The behavior modification theory will be applied in the form of a structured set of cues and questions between investigator and participant at each visit in order to provide individually tailored counseling to enhance internal motivation and facilitate behavior change toward physical activity and dietary improvement that are adaptable to each participant's usual habits.

Follow Up
The study will occur over a total of 14 weeks, although duration of the intervention will consist of 12 weeks. The first two weeks will serve the purpose of identifying the baseline physical activity of each participant. During the second week all baseline studies/determinations will be completed, with randomization occurring by the end of week 2. Allocation will be stratified by presence or absence of refractory ascites.

Follow up visits for behavioral therapy will occur every 2 weeks for the first 8 weeks, and then on a 4-weekly basis (only active group). Nutritional consultation will occur every 2 to 4 weeks throughout the study (both active and control group). During each visit, the EAA supplement jar will be inspected for percent emptiness (in analogy to a pill count) in order to quantify adherence. Bi-weekly phone calls will be made to all subjects to enhance adherence to recommendations. Standard of care investigations (blood chemistries, hematology and coagulation, liver function tests, etc.) will be ordered according to the needs of the patient and the criteria of the investigator or responsible clinician. Clinical status and complications from portal hypertension, particularly variceal bleeding and HE, will be systematically investigated on each visit.

Research Tests - Variables to Measure
Prior to randomization each subject will be scheduled to undergo all baseline investigations, as follows (see Research Calendar below):

Cardiorespiratory stress test (CRET) and anaerobic threshold (Week -1, Day 1)
CRET will be performed early in the morning on a fasting state, following the maximal incremental cycle ergometry protocol from our Pulmonary Physiology Laboratory, and in agreement with the American Thoracic Society.(38) Briefly, test consists on having the patient on a cycle ergometer performing a progressively increasing work rate until reaching: volitional exhaustion, or termination by the medical monitor. The Borg Rating of Perceived Exertion Scale will be used to help titrate intensity of work rate (aiming for a level of 13 to 17). Gas exchange measurement for

oxygen uptake and carbon dioxide outflow will be performed during the test, in order to determine VO_2 (oxygen consumption) and VCO_2 (carbon dioxide consumption) in real time. The VO_{2max} will be identified as a clear plateau in the VO_2 curve. In cases where no plateau can be observed due to exhaustion, the VO_{2peak} at the time of test termination will be used as an equivalent. The anaerobic threshold will be identified by means of the V-slope method on the VO_2 to VCO_2 plot (respiratory exchange ratio).(38) Thirty minutes after completion of the test, each patient will be clinically evaluated for clinical signs of HE according to standard criteria (Table 1 and Figure 2). This test will be repeated after completion of the study period (12 weeks), and 6 months after LT.

Ammonia and lactate thresholds (Week -1, Day 1)
In order to obtain frequent venous samples, an 18-20 Ga IV catheter with a saline lock will be placed in the forearm before the test. Ammonia levels will be determined on venous samples with an automated point-of-care device (PocketChem Ammonia Checker II, Arkray Inc., Kyoto, Japan), prior to the start of the test, and then repeated every 3 minutes until termination of test, with a final sample 20 minutes after termination of the test. This will allow identification of the ammonia threshold when results are plotted against % VO_{2max}. After a baseline determination of lactate, repeated samples will be obtained every 2 minutes, immediately after the test is finished, and 20 min after termination. This will allow correlating the anaerobic threshold obtained from the respiratory exchange ratio with the lactate threshold. For both the ammonia and lactate thresholds is it estimated that 6-7 blood draws will be performed per patient (4 mL per sample) corresponding to 24-28 mL in total. These two tests will be repeated after completion of the study period (12 weeks).

Six-minute walk test (6MWT) (Week -1, Day 3)
The 6MWT will be performed in agreement with the recommendations of the American Thoracic Society.(39) In brief, the test will be done indoors, along a long and flat enclosed corridor, aiming for as many laps (100 ft per half-lap) as possible, while patient is wearing a pulse oxymeter. The investigator applying the test will use a stopwatch and write down heart rate and oxygenation, at baseline, and every minute until reaching a total of six minutes. The Modified Borg Scale will be used to rate shortness of breath and fatigue before and after the test. This test will be repeated after completion of the study period (12 weeks), and 6 months after LT.

Sarcopenia (abdomen CT-scan) (Week -1, Day 3)
A noncontrasted abdominal CT-scan will be used to measure the cross-sectional area of skeletal muscle at the level of the third lumbar vertebrae (L3). Images will be analyzed with SliceOmatic 4.3 (Tomovision, Montreal, Canada) measuring the cross-sectional area of skeletal muscle at this level (*i.e. psoas, paraspinals, quadratus lumborum, transversus abdominis, rectus abdominis,* and *internal* and *external obliques*). Muscle areas will be analyzed by an operator trained in musculoskeletal anatomy with tissue-specific Hounsfield unit thresholds (-29 to +150).(16) The L3 skeletal muscle area will be normalized to stature by the division of the muscle area by the height squared. Sarcopenia will be defined as a reduction in the L3 area of muscle mass ≤52.4 cm^2/m^2 in males and ≤38.5 cm^2/m^2 in females. This test will be repeated after completion of the study period (12 weeks), and 6 months after LT.

Nutritional consultation, anthropometrics, and body composition (Week -2, Day 3)
A dietitian or designated investigator will evaluate anthropometrics (weight, height, waist circumference), bioelectrical impedance, and whole-body dual energy X-ray absorptiometry scan (DXA). Bioelectrical impedance will be performed according to the manufacturer's recommendations: after overnight fasting, with subject in supine position with arms and legs abducted from the body, and source and sensor electrodes placed on the dorsum of both hand and foot on the right side of the body, respectively. After obtaining resistance (R), reactance (Xc)

and phase angle (PhA), two analyses will be performed: PhA and bioelectrical impedance vector analysis (BIVA). PhA will be evaluated as a nutritional marker reflecting muscle mass. Sarcopenia will be defined as a PhA < 5.4°. Following normalization of R and Xc to patient´s height (R/H, and Xc/H, respectively), R/H and Xc/H will be plotted as bivariate vectors on the R-Xc graph (expressed in Ohm/m) in order to obtain BIVA. Individual vectors for each patient will be calculated and analyzed to determine nutritional status, muscle mass, and hydration status. For DXA a standard protocol and quality assurance procedure will be followed. DXA will be used to estimate body composition. Briefly, before each DXA scan, the subjects' height, weight, gender, and race will be entered into the computer program. The subjects will be in a supine position on the DXA table with hands pronated and flat on the table. Total body mode will be selected for each scan, and scanning thickness will be determined by the DXA software. Anthropometrics, PhA, BIVA, and DXA will be repeated after completion of the study period (12 weeks), and 6 months after LT.

Blood Biomarkers
Myostatin and IGF-1 levels will be determined in blood (R&D Systems, Minneapolis, MN) at baseline and during week 12 visit to clinic. Also, at baseline and during week 12 visit blood EAA (high-performance liquid chromatography; vendor, City, State) will be quantified. The latter will be performed at Dr. Arny Ferrando's laboratory at no cost. Dr. Ferrando will be blinded to the source of serum samples. In order to facilitate blinding, blood samples will be processed by clinical coordinator into aliquots, de-identified, and stored at Dr. Ferrando's laboratory for future assay running. Only the clinical coordinator and the PI will have access to the code that identifies the serum samples provided for EAA quantification.

Quality of life and metabolic profile (Week -2, Day 3)
The questionnaire Sickness Impact Profile (SIP) will be used to assess changes in the quality of life. Recent evidence suggests SIP to be superior to SF-36 in detecting health-related quality of life changes occurring in patients with ESLD. Metabolic profile will include determinations of fasting glucose, insulin (in subjects without diabetes mellitus), lipid profile (triglycerides, total-cholesterol, HDL-cholesterol, LDL-cholesterol), and HbA1c. Both SIP and the metabolic profile will be repeated after completion of the study period (12 weeks). The metabolic profile will be repeated 6 months after LT as well.

Randomization
Each participant will be randomly assigned to the active (physical activity + behavioral therapy + nutritional intervention) or control (nutritional intervention) group. The random allocation sequence will be generated using computer-generated random numbers. There will be no stratification in the randomization process, and allocation will be concealed.

Statistical analysis
Subjects sample has been defined by convenience, as there is no precedent for sample estimation. Descriptive statistics will include mean ± SD and/or median (range), according to distribution of data. Shapiro-Wilk test will be used to assess normal distribution. Results on VCO_2 will be plotted against VO_2, whereas lactate, and ammonia will be plotted against VO_{2max}, for all patients as an aggregate. The lines determining the anaerobic, lactate, and ammonia thresholds will be calculated by linear regression. Continuous variables comparisons within groups will be done with paired t-test or Wilcoxon, whereas between groups will be done with t-test or Mann-Whitney. Categoric variables will be compared with chi-square or Fisher's exact test, as appropriate. Agreement between methods used to address body composition, bioelectrical impedance and DXA, will be investigated with Bland-Altman test.

Data collection and handling

Apart from the data generated by the study demographics and clinical information to properly classify the hepatic functional and cardiorespiratory status of each participant will be retrieved from the electronic medical record (EMR). Such information will include results from standard of care laboratories, imaging, and other special studies (EGD, pulmonary function tests, echocardiogram), to calculate MELD score, and Child-Turcotte-Pugh scores.

A database will be created to accumulate all of the clinical information generated during this study. No identifiers, apart from name and DOB, will be collected. All data will be stored in databases kept in a study folder created at PI's computer (password-protected). Main database will include aforementioned identifiers, however, each subject will have a unique study ID assigned, and once an analytic table is created, all PHI will be removed from the table (after approval by the PI). Only this de-identified dataset with unique study ID's will be used for statistical analyses. Therefore, the risk to the privacy of the individuals will be minimized.

The access to the research folder and its contents will be restricted to the research staffs listed in this submission form. The PHI will be kept in the main database until completion of the study. The main database containing PHI will be kept in the study folder and will be never transferred out without de-identification of the data. No hard copy research data will be generated in this project.

In order to quantify the muscle mass at the level of L3, electronic copies of abdomen CT-scans with no identifiers will be shared with Aldo Montano-Loza from the University of Alberta, in Canada. After proper quantification an analytic table including unique study ID's will be sent back to UAMS for integration into the main database.

Risks and Benefits
CRET is a safe procedure and the risk for having a complication, such as an acute coronary syndrome is mostly related to underlying chronic disease. The risk of death varies as well, from 1 death in 20,000 - 70,000 tests, depending mostly on the presence of underlying heart disease. In cohorts including healthy subjects, no deaths or major complications have been observed. Since LT candidates have to undergo a thorough cardiopulmonary evaluation and patients with any moderate to severe cardiopulmonary condition are not considered transplant candidates the risks for any major complication or death in this study should equal zero. Moreover, the personnel taking care of CRET constantly monitors physiologic responses to exercise (including electrocardiogram monitoring), and are trained to stop the test whenever there are signs for an impending complications, and can immediately and effectively perform cardiac resuscitation.

Unlike CRET, in the 6MWT each patient determines the intensity of their exercise according to symptoms and cardiorespiratory fitness. The 6MWT is thus considered a very safe procedure, and it has been performed (without electrocardiogram monitoring) in thousands of older persons and thousands of patients with heart failure or cardiomyopathy without serious adverse events. Although patients with cardiopulmonary disease may be at increased risk for arrhythmias or cardiovascular collapse during testing, it is very unlikely for a complication to be witnessed given the selection criteria followed during LT evaluation (as above).

The risks associated with placement of an IV catheter and blood collection from a vein are minor bleeding, bruising, infection (a small risk any time the skin is broken), or needing more than one attempt to locate veins.

Both CT and DXA scans are associated with exposure to small amounts of ionizing radiation. The abdominal CT-scan will be restricted to a simple phase (non-contrasted) in order to limit radiation exposure, and thus it should not exceed 10 mSv. Since the initial CT-scan is performed as part

of the LT evaluation (standard of care), the study will derive in radiation exposure of approximately 20 mSv. This is a very low radiation exposure that might be associated with a low additional increase in lifetime risk for cancer (1 in 1000 to 10,000). In the case of DXA, exposure per whole-body scan is similar to daily background natural radiation exposure (0.01 mSv).

The electrical current used in bioelectrical impedance is very small and thus unlikely to stimulate electrically excitable tissues, particularly cardiac and nervous tissues. Moreover, there are no reports of untoward effects among thousands of individuals in whom bioelectrical impedance analysis has been performed. Actually, the currents employed are very small and below the threshold of perception.

No complication is properly expected from the exercise program since each patient will tailor her/his own intensity of exercise according to individual fitness and symptoms that might occur while exercising (i.e. fatigue, or shortness of breath). Although there is a theoretical increased risk for variceal bleeding and HE, these complications have not been observed in prior studies. Moreover, the CRET and ammonia and lactic acid determinations (obtained with ammonia/lactate thresholds) would provide the investigator with some sense of risk for complications during intense exercise and could adequately advise patients at a higher risk for complications to avoid moderate to intense exercise.

The database containing PHI will remain within UAMS firewall and only used for specific research purposes in this project. Aggregated data will be analyzed and published, but specific data elements will not be made available. Further, subjects will not forfeit any rights by participating - their health care and eligibility for health benefits will not be affected in any way. Although a breach in confidentiality is always a risk when collecting personal information from patients, we believe this is small given the system that has been developed to obtain and handle data.

All participants might benefit from study by increasing their physical fitness and dietary habits during and after the study, and they will be able to keep their Fitbit to continue monitoring physical activity.

Ethics

This study will be conducted according to US and international standards of Good Clinical Practice (FDA regulations) for all studies. Applicable government regulations, University of Arkansas for Medical Sciences research policies and procedures will also be followed. This protocol and any amendments will be submitted and approved by the University of Arkansas for Medical Sciences Institutional Review Board (IRB) to conduct the study. 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 formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

Figure 1. Plots showing VO_{2max}, anaerobic, and lactate/ammonia thresholds.

1a **1b** **1c**

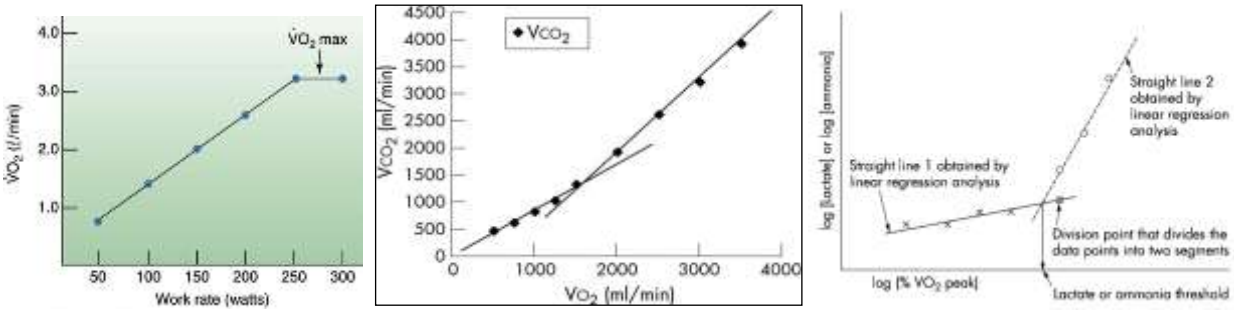

Figure 2. Relationship between SONIC and West Haven (WH) criteria.

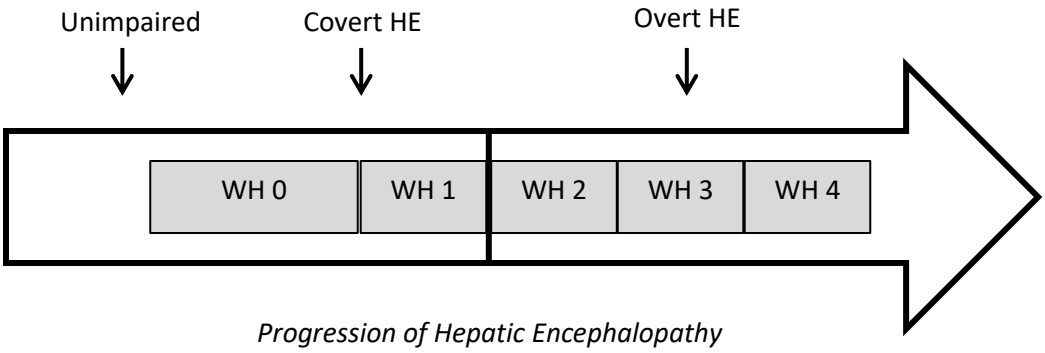

Table 1. West Haven criteria for diagnosis and classification of overt hepatic encephalopathy.

West Haven	Features
Grade 0	No abnormalities detected
Grade 1	Trivial lack of awareness Euphoria or anxiety Shortened attention span Impairment of addition or subtraction
Grade 2	Lethargy or apathy Disorientation for time Obvious personality change Inappropriate behavior
Grade 3	Somnolence to semi-stupor Response to stimuli Confused Gross disorientation Bizarre behavior
Grade 4	Coma, unable to test mental state

BIBLIOGRAPHY

1. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. Journal of hepatology. 2006;44(1):217-31.
2. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep. 1985;100(2):126-31.
3. Tudor-Locke C, Hatano Y, Pangrazi RP, Kang M. Revisiting "how many steps are enough?". Medicine and science in sports and exercise. 2008;40(7 Suppl):S537-43.
4. Konishi I, Hiasa Y, Tokumoto Y, Abe M, Furukawa S, Toshimitsu K, et al. Aerobic exercise improves insulin resistance and decreases body fat and serum levels of leptin in patients with hepatitis C virus. Hepatology research : the official journal of the Japan Society of Hepatology. 2011;41(10):928-35.
5. Pattullo V, Duarte-Rojo A, Soliman W, Vargas-Vorackova F, Sockalingam S, Fantus IG, et al. A 24-week dietary and physical activity lifestyle intervention reduces hepatic insulin resistance in the obese with chronic hepatitis C. Liver international : official journal of the International Association for the Study of the Liver. 2013;33(3):410-9.
6. Soriano G, Roman E, Cordoba J, Torrens M, Poca M, Torras X, et al. Cognitive dysfunction in cirrhosis is associated with falls: a prospective study. Hepatology. 2012;55(6):1922-30.
7. Lemyze M, Dharancy S, Wallaert B. Response to exercise in patients with liver cirrhosis: implications for liver transplantation. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2013;45(5):362-6.
8. Montano-Loza AJ, Duarte-Rojo A, Rose C. Sarcopenia and myosteatosis increase the risk of hepatic encephalopathy in cirrhotic patients. Hepatology. 2014;60(4):384-5A.
9. Ruiz-Margain A, Macias-Rodriguez RU, Duarte-Rojo A, Espinosa-Cuevas A, Torre A. Association between malnutrition in patients with liver cirrhosis and the presence of hepatic encephalopathy. Hepatology. 2014;60(4):380A.
10. Qiu J, Tsien C, Thapalaya S, Narayanan A, Weihl CC, Ching JK, et al. Hyperammonemia-mediated autophagy in skeletal muscle contributes to sarcopenia of cirrhosis. American journal of physiology Endocrinology and metabolism. 2012;303(8):E983-93.
11. Dharancy S, Lemyze M, Boleslawski E, Neviere R, Declerck N, Canva V, et al. Impact of impaired aerobic capacity on liver transplant candidates. Transplantation. 2008;86(8):1077-83.
12. Tandon P, Ney M, Irwin I, Ma MM, Gramlich L, Bain VG, et al. Severe muscle depletion in patients on the liver transplant wait list: Its prevalence and independent prognostic value. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 2012;18(10):1209-16.
13. Carey EJ, Steidley DE, Aqel BA, Byrne TJ, Mekeel KL, Rakela J, et al. Six-minute walk distance predicts mortality in liver transplant candidates. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 2010;16(12):1373-8.
14. Englesbe MJ, Patel SP, He K, Lynch RJ, Schaubel DE, Harbaugh C, et al. Sarcopenia and mortality after liver transplantation. Journal of the American College of Surgeons. 2010;211(2):271-8.
15. Neviere R, Edme JL, Montaigne D, Boleslawski E, Pruvot FR, Dharancy S. Prognostic implications of preoperative aerobic capacity and exercise oscillatory ventilation after liver transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2014;14(1):88-95.
16. Montano-Loza AJ, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, et al. Muscle wasting is associated with mortality in patients with cirrhosis. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2012;10(2):166-73, 73 e1.

17. Montano-Loza AJ, Duarte-Rojo A, Meza-Junco J, Baracos VE, Sawyer MB, Pang JX, et al. Inclusion of Sarcopenia Within MELD (MELD-Sarcopenia) and the Prediction of Mortality in Patients With Cirrhosis. Clin Transl Gastroenterol. 2015;6:e102.
18. Durand F, Buyse S, Francoz C, Laouenan C, Bruno O, Belghiti J, et al. Prognostic value of muscle atrophy in cirrhosis using psoas muscle thickness on computed tomography. Journal of hepatology. 2014;60(6):1151-7.
19. Lemyze M, Dharancy S, Neviere R, Pruvot FR, Declerck N, Wallaert B. Aerobic capacity in patients with chronic liver disease: Very modest effect of liver transplantation. Presse Med. 2010;39(7-8):e174-81.
20. Kouz J, Vincent C, Leong A, Dorais M, Rakel A. Weight gain after orthotopic liver transplantation: Is nonalcoholic fatty liver disease cirrhosis a risk factor for greater weight gain? Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 2014;20(10):1266-74.
21. Pagadala M, Dasarathy S, Eghtesad B, McCullough AJ. Posttransplant metabolic syndrome: an epidemic waiting to happen. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 2009;15(12):1662-70.
22. Wing RR, Bolin P, Brancati FL, Bray GA, Clark JM, Coday M, et al. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. The New England journal of medicine. 2013;369(2):145-54.
23. Goldberg RB, Mather K. Targeting the consequences of the metabolic syndrome in the Diabetes Prevention Program. Arteriosclerosis, thrombosis, and vascular biology. 2012;32(9):2077-90.
24. Agarwal A, Pattullo V, Garcia-Saenz-de-Sicilia M, Sockalingam S, Fantus IG, Allard J, et al. Can a lifestyle intervention improve risk factors for a negative response to peginterferon treatment in patients with chronic hepatitis c, cirrhosis, and obesity? Journal of hepatology. 2014;60:S478.
25. Soriano GR, E.; Torrades, M.T.; Cardenas, G.; Cordoba.; Vidal, S. Impact of an exercise programme on quality of life, effort tolerance and muscle mass in patients with cirrhosis. Journal of hepatology. 2011;54:S61.
26. Dasarathy S. Cause and management of muscle wasting in chronic liver disease. Curr Opin Gastroenterol. 2016;32(3):159-65.
27. Lemyze M, Dharancy S, Neviere R, Wallaert B. Cardiopulmonary response to exercise in patients with liver cirrhosis and impaired pulmonary gas exchange. Respiratory medicine. 2011;105(10):1550-6.
28. Pieber K, Crevenna R, Nuhr MJ, Quittan M, Peck-Radosavljevic M, Fialka-Moser V, et al. Aerobic capacity, muscle strength and health-related quality of life before and after orthotopic liver transplantation: preliminary data of an Austrian transplantation centre. Journal of rehabilitation medicine : official journal of the UEMS European Board of Physical and Rehabilitation Medicine. 2006;38(5):322-8.
29. Wiesinger GF, Quittan M, Zimmermann K, Nuhr M, Wichlas M, Bodingbauer M, et al. Physical performance and health-related quality of life in men on a liver transplantation waiting list. Journal of rehabilitation medicine : official journal of the UEMS European Board of Physical and Rehabilitation Medicine. 2001;33(6):260-5.
30. Wong F, Girgrah N, Graba J, Allidina Y, Liu P, Blendis L. The cardiac response to exercise in cirrhosis. Gut. 2001;49(2):268-75.
31. Garcia-Pagan JC, Santos C, Barbera JA, Luca A, Roca J, Rodriguez-Roisin R, et al. Physical exercise increases portal pressure in patients with cirrhosis and portal hypertension. Gastroenterology. 1996;111(5):1300-6.
32. Bandi JC, Garcia-Pagan JC, Escorsell A, Francois E, Moitinho E, Rodes J, et al. Effects of propranolol on the hepatic hemodynamic response to physical exercise in patients with cirrhosis. Hepatology. 1998;28(3):677-82.
33. Macias-Rodriguez RU, Torre A, Ilarraza-Lomeli H, Ruiz-Margain A, Garcia-Flores O, Duarte-Rojo A. Changes in hepatic venous pressure gradient induced by a phsyical exercise program in cirrhotic patients: a randomized open clinical trial. Hepatology. 2014;60(4):246-7A.

34. Rose CF. Ammonia-lowering strategies for the treatment of hepatic encephalopathy. Clinical pharmacology and therapeutics. 2012;92(3):321-31.
35. Yuan Y, So R, Wong S, Chan KM. Ammonia threshold--comparison to lactate threshold, correlation to other physiological parameters and response to training. Scandinavian journal of medicine & science in sports. 2002;12(6):358-64.
36. Yuan Y, Chan KM. A longitudinal study on the ammonia threshold in junior cyclists. British journal of sports medicine. 2004;38(2):115-9.
37. Totti V, Zancanaro M, Trerotola M, Nanni Costa A, Antonetti T, Anedda A, et al. Quality of life and energy expenditure in transplant recipient football players. Transplantation proceedings. 2013;45(7):2758-60.
38. ATS/ACCP Statement on cardiopulmonary exercise testing. American journal of respiratory and critical care medicine. 2003;167(2):211-77.
39. ATS statement: guidelines for the six-minute walk test. American journal of respiratory and critical care medicine. 2002;166(1):111-7.

STUDY CALENDAR

	Basal	Wk -2	Wk -1	Wk 0	Wk 2	Wk 4	Wk 6	Wk 8	Wk 12	Post-LT
Sign consent	✓									
CRET			✓						✓	✓
Anaerobic threshold			✓						✓	✓
6MWT			✓						✓	✓
NH3 & Lactate thresholds			✓						✓	
CT scan			✓						✓	✓
SIP		✓							✓	
Bioimpedance & DXA		✓							✓	✓
Nutritional consultation				✓	✓	✓	✓	✓		
Pill count					✓	✓	✓	✓	✓	
Anthropometry		✓						✓	✓	✓
Pedometer count		✓		✓	✓	✓	✓	✓	✓	
Heart rate count		✓		✓	✓	✓	✓	✓	✓	
Cognitive Therapy					✓	✓	✓	✓		
Metabolic profile	✓								✓	✓
Myostatin & IGF-1			✓						✓	
Essential amino acids			✓						✓	

Cognitive therapy will be provided only to the active group.
IGF-1: insulin-related growth factor 1