UNIVERSITY OF OREGON

College of Arts and Sciences

Study Title: Mechanistic Approach to Preventing Atrophy and Restoring Function in Older Adults

NCT number: NCT02145949

Date of the document: January 26, 2019.

DEPARTMENT OF HUMAN PHYSIOLOGY

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DATE: January 26, 2019 **IRB Protocol Number: 12272013.024**

TO: Hans Dreyer, Principal Investigator
Department of Human Physiology

RE: Protocol entitled, "Mechanistic approach to preventing atrophy and restoring function in older adults"

Notice of IRB Review and Approval
Amendment Review

The amendment submitted on January 04, 2019 for the project identified above has been reviewed and approved by the Committee for Protection of Human Subjects (CPHS), the University of Oregon Institutional Review Board (IRB). The IRB has approved the changes to the research as described in the attached materials. As a reminder, it is your responsibility to submit any proposed changes for IRB review and approval prior to implementation.

Amendment Description:

- Separated inclusion and exclusion criteria for patients who are eligible and interested in having biopsies vs. patients who are ineligible due to timeline or are not within the biopsy window.
- Revised the pre-operative study timeline from -6 weeks (+/- 2 weeks) to -8 weeks to –1 week.
- Revised the informed consent to reflect the timeline requirements and option to participate in biopsies, as well as the option to participate in the study in the timeline for biopsies is too short (<4 weeks from date of surgery).
- Revised the Research Plan and consent form accordingly.

For this research, the following determinations have been made:

- **This research was previously determined to be greater than minimal risk and requires full board review.**
- **This amendment was determined to be a minor change to previously approved research and was reviewed in accordance with expedited review procedures as per Title 45 CFR 46.110 under the pre-2018 Common Rule.**

Approval period: January 26, 2019 - November 06, 2019

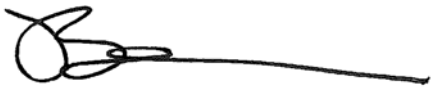
If you anticipate the research will continue beyond the IRB approval period, you must submit a **Continuing Review Application** to request continued approval at least 45-days prior to the expiration date. **Without continued approval, the protocol will expire on November 06, 2019 and human subject research activities must cease.** A closure report must be submitted once human subject research activities are complete. Failure to maintain current approval or properly close the protocol constitutes non-compliance.

You are responsible for adhering to the *Investigator Agreement* submitted with the initial application for IRB review. The responsibilities of the agreement are reiterated at the end of

this letter below. You are responsible for conduct of the research and must maintain oversight of all research personnel to ensure compliance with the IRB approved protocol.

The University of Oregon and Research Compliance Services appreciate your commitment to the ethical and responsible conduct of research with human subjects.

Sincerely,



Daniel Berman
Research Compliance Administrator

CC: Tessa Kirkpatrick, Erin Owen

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INVESTIGATOR AGREEMENT

Principal Investigator and Faculty Advisor Responsibilities

A. Conduct of the Research

1. I accept responsibility for the ethical conduct of this research and protection of participants as set forth in the Belmont Report, Declaration of Helsinki, the Nuremberg Code, the Common Rule, and the ethical principles of my discipline.
2. I accept responsibility for the conduct of this research ensuring this research is conducted according to
    a. sound research design and methods;
    b. the IRB approved protocol including the informed consent process;
    c. the applicable terms of the grant, contract and/or signed funding agreements; and
    d. applicable laws and regulations, including those for protecting the rights, safety, and welfare of human subjects.
3. I certify that I am or my faculty advisor is sufficiently qualified by education, training, and/or experience to assume responsibility for the proper conduct of this research. I accept responsibility for ensuring that members of this research team, including study staff and trainees, are appropriately qualified, trained and supervised.
4. I accept responsibility to personally conduct and/or directly supervise this research. I certify that I have sufficient time and resources to properly conduct and/or supervise this research for which I am responsible.

B. Ensuring and Maintaining Compliance

1. I will comply with relevant regulatory and institutional requirements, including those relating to conflicts of interest, responsible conduct of research and research misconduct.
2. I understand it is my responsibility to ensure that any research personnel, including myself, responsible for the design, conduct, and reporting of research declare any potential conflicts of interests related to the research and to maintain current records. I will ensure changes in conflicts of interest are promptly disclosed to the IRB.
3. I will ensure that informed consent is obtained as approved by the IRB and a copy is provided to participants, unless the IRB waives these requirements.
4. I will obtain initial IRB approval prior to implementing human subject research activities as well as prior approval for any amendments to this research.
5. I will conduct this research within the approval period issued by the IRB. I agree to submit a request for continuing review of this research at least 45 days in advance of the expiration date.
6. I will submit a closure report form prior to protocol expiration or within 45 days of completion of all activities involving human subjects or identifiable participant data.
7. I will maintain approval, as applicable, with collaborative entities including approvals from other countries or jurisdictions.
8. I will promptly report to the IRB (no later than seven days of discovery) any instances of noncompliance with the approved protocol or requirements of the IRB and any unanticipated problems.

INVESTIGATOR AGREEMENT

Principal Investigator and Faculty Advisor Responsibilities

9. I will assist in the facilitation of any monitoring and/or auditing of study activities and/or records as required by the IRB, funding entities, sponsors, and any federal and state regulatory agencies.

C. Investigator Records, Reports and Documentation

1. I will maintain research records, all protocol materials, and any other documents associated with this research (e.g., research plan, signed consent forms, and IRB correspondence).
2. I will maintain records for at least three years after this research ends, or for the length of time specified in applicable regulations or institutional or sponsor requirements, whichever is longer. I will take measures to prevent accidental or premature destruction of these documents.
3. I will ensure the safe and secure storage of this research data (whether in paper or electronic formats) and for protecting the confidentiality of the data in accordance with the approved protocol.
4. I will submit written reports to the IRB and permit inspection of the research records as required by the IRB.

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COMMITTEE FOR THE PROTECTION OF HUMAN SUBJECTS
RESEARCH COMPLIANCE SERVICES

AMENDMENT APPLICATION

Purpose: This application is designed to help facilitate review of changes to an existing protocol and to assure compliance of the federal regulations as set forth in 45 CFR Part 46.

Instructions: Use this application to request IRB review of proposed changes to **previously approved expedited or full board** research. You must obtain IRB approval **prior** to implementing any change(s) in your research. This application can also be used to request a study previously reviewed under the pre-2018 Common Rule regulations be considered for transition to oversight under the 2018 Common Rule. Submit this application and all applicable research materials solicited in the checklist at the end of the form to ResearchCompliance@uoregon.edu. Save this form before proceeding.

PART I: STUDY AND INVESTIGATOR INFORMATION

Principal Investigator (PI):	Hans C. Dreyer, PhD, PT	Today's Date:	1/4/2019
Faculty Advisor		Protocol number:	12272013.024
Study Title:	Mechanistic approach to preventing atrophy and restoring function in older adults		

Research Status (check one)

- ☐ Project not yet started (no subjects being recruited)
- ☒ Currently in progress (subjects being recruited)
- ☐ Closed to new subject entry (long term follow-up only or data analysis)

PART II: PROPOSED CHANGES TO PREVIOUSLY APPROVED RESEARCH

1. Give a brief description of the proposed change(s).

Separation of inclusion and exclusion criteria for patients who are eligible and interested in having biopsies vs. patients who are ineligible due to timeline, or are not within biopsy window. We have separated the inclusion/exclusion criteria into two categories: Biopsy participant and non-biopsy participant.

The pre-operative study timeline has been updated from -6 weeks (+/- 2 weeks) to -8 weeks to -1week to account for the ability of patients to enroll before supplement ingestion as long as there is enough time to complete pre-operative study activities, including completion of biopsies at least 4 weeks prior to date of surgery.

Changes to the informed consent document reflect the timeline requirements and option to participate in biopsies, as well as option to participate in the study if the timeline for the biopsies is too short (< 4 weeks from date of surgery).

Last, the final consent page in the informed consent document has separated two of the existing bullets into two separate bullets for clarity.

2. Describe the rationale for the change(s).

The current protocol allows for patient to opt in or out of biopsies. However, the inclusion and exclusion criteria are structured to account for risks associated with biopies. We have divided out the inclusion and exclusion criteria to

UNIVERSITY OF OREGON

COMMITTEE FOR THE PROTECTION OF HUMAN SUBJECTS
RESEARCH COMPLIANCE SERVICES

AMENDMENT APPLICATION

reflect patients who are opting in and/or eligible for biopsy consideration versus patients who opt out, or due to timeline, are not eligible for biopsies.

Timeline: Patients who are less than 4 weeks from the date of surgery are not eligible for biopsies, but could be included in study and not undergo any biopsies.

Biopsy risk: Patients who are timeline eligible (more than 4 weeks from date of surgery) and opt to have biopsies performed, need to be evaluated for bleeding risk associated with taking an oral blood thinner medication. The current exclusion criteria excludes patients taking oral blood thinners. Patients who choose not to participate in the biopsies or who are not within study window for biospies (ineligible for biospies due to proximity to the date of surgery) can still enroll in the study, but do not need to be evaluated for bleeding risk secondary to taking oral blood thinners.

3. Are you making changes to your previously established project period at this time?

☒ Yes ☐ No | Provide anticipated end date for human subject research activities (month and year):

PART III: RESEARCH PERSONNEL

If you are you making any changes to research personnel at this time (e.g., adding research staff, changing PI, etc.), list the individuals below and attach an updated Research Personnel Form highlighting those individuals.

☒ No changes to Research Personnel; proceed to Part IV below.

☐ Research Personnel Form is attached; the following individuals have been added and/or updated:

- It is the responsibility of the Principal Investigator (PI) to ensure that any research personnel, including the PI, *responsible for the design, conduct, and reporting of research* complete the Human Subjects Conflict of Interest (COI) form.
- The PI must keep completed copies of all Human Subject COI forms for their records.
- The PI must submit with this application Human Subject COI forms only for
  - New research personnel who have identified a real, perceived, or potential conflict of interest on their form; and
  - Existing personnel who have identified a change to a real, perceived, or potential conflict of interest on their form.

☒ No conflicts are identified.

☐ Yes, conflicts and/or changes are identified and Human Subject COI form(s) are attached for the following individuals:

PART IV: RESEARCH RISK

1. Based on the proposed change(s), are there any new or altered risks?

☐ Yes ☒ No Explain in the text box below:

Patients participating in biospies cannot be taking oral blood thinners to participate; patients not having biopsies may be taking oral blood thinners at the time of enrollment, as the risk from taking oral blood thinners is the risk of bleeding from biopsies.

2. In your opinion, how do the proposed change(s) impact the overall risk profile for the previously approved research (select one of the following):

☐ Increase ☐ Decrease ☒ Remain the same

Provide rationale and justification with support for your response.

Addressed in response to Part IV, question 1.

3. Is the proposed amendment a result of an unanticipated problem or adverse event?

☐ Yes ☒ No If "Yes", explain in the text box below

4. As a result of the proposed change(s), has a Data and Safety Monitoring Plan (DSMP) been created for this research? This is typically required by a sponsor or regulatory agency (e.g., FDA).

☐ Yes ☒ No If "Yes," attach a copy of the DSMP and address in the Research Plan.

5. As a result of the proposed change(s), has a Data Safety Monitoring Board or Committee (DSMB/DSMC) been established for this research? This is typically required by a sponsor or regulatory agency (e.g., FDA).

☐ Yes ☒ No If "Yes," attach a copy of the DSMB/DSMC information and address in the Research Plan.

PART V: COLLABORATIONS

1. As a result of the proposed change(s), will the research be conducted with institutions, or at site(s)/organization(s) other than University of Oregon (e.g., public schools, tribes, non-profit organizations, companies, hospitals, universities, etc.)?

☐ Yes ☒ No If "Yes", explain and attach applicable permission and/or approval documentation:

PART VI: MATERIALS

1. New and revised materials must be submitted with this application. Revisions to any previously approved protocol materials must be submitted with proposed changes clearly indicated (e.g., using track changes).

Check material type below and indicate if this is new or revised.

Material Type			New	Revised	Title(s)/Comments/Other
☐	Form - Personnel		☐	☐	
☐	Form - Conflict of Interest		☐	☐	
☐	Form - Funding		☐	☐	
☒	Research Plan		☐	☒	
Incl.	n/a				
☐	☐	Appendix A - Drugs	☐	☐	
☐	☐	Appendix B - Medical Devices	☐	☐	
☐	☐	Appendix C - Ionizing Radiation	☐	☐	
☐	☐	Appendix D - HIPAA	☐	☐	
☐	☐	Appendix E - Genetic Materials	☐	☐	
☐	Recruitment Materials		☐	☐	

AMENDMENT APPLICATION

Material Type		New	Revised	Title(s)/Comments/Other
☒	Consent/ Assent Materials	☐	☒	
☐	Debriefing Materials			
☐	Data Collection Materials/Instruments	☐	☐	
☐	Data Safety Monitoring Plan			
☐	Data Safety Monitoring Board/Committee Information	☐	☐	
☐	Permissions/ Support Letters/ Outside IRB Approval	☐	☐	
☐	For funded/sponsored Research: Human Subjects portion of grant Proposal	☐	☐	
☐	Other (specify)	☐	☐	
☐	Other (specify)	☐	☐	
☐	Other (specify)	☐	☐	

2. As a result of the proposed changes, will any of the previously approved protocol materials no longer be used?

☐ Yes ☒ No If "Yes", list materials below

	Material Name/Type/Title/Comments
☐	
☐	
☐	
☐	
☐	
☐	

[Remainder of page intentionally left blank; acknowledgements and signature page to follow.]

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COMMITTEE FOR THE PROTECTION OF HUMAN SUBJECTS
RESEARCH COMPLIANCE SERVICES

AMENDMENT APPLICATION

PART VII: INVESTIGATOR AND FACULTY ADVISOR SIGNATURES

- By signing below I certify that I will conduct this research as approved by the University of Oregon CPHS (IRB) and in accordance with the Investigator Agreement.
- I understand that any changes listed above may not be implemented in the human subjects research until this amendment has been approved by the CPHS (IRB).

Hans C. Dreyer, PhD, PT	January 4, 2019
Principal Investigator Signature	**Date**

- *Electronic signatures acceptable. The name of the Principal Investigator may be typed in the signature line.*
- *If the person emailing this application is not the Principal Investigator, the Principal Investigator must be copied on this application submission.*

REQUIRED FOR STUDENT RESEARCH

- By signing this form, the Faculty Advisor attests that (s) he has reviewed the proposed change and agrees to provide appropriate education, oversight, and supervision of the student investigator above, and share the above Principal Investigator responsibilities.

Click here to type name or insert electronic signature.	Click here to enter a date.
Faculty Advisor Signature	**Date**

- *Electronic signatures acceptable. The name of the Faculty Advisor may be typed in the signature line.*
- *If the person emailing this application is not the Faculty Advisor, the Faculty Advisor must be copied on this application submission.*

RESEARCH PLAN

MECHANISTIC APPROACH TO PREVENTING ATROPHY AND RESTORING FUNCTION IN OLDER ADULTS

National Institute on Aging: R01AG046401-01A1

PRINCIPAL INVESTIGATOR
Hans C. Dreyer, PhD, PT

Co-Investigators
Brian A. Jewett, MD
Brick A. Lantz, MD
Craig G. Mohler, MD
Steven N. Shah, MD

UO IRB Protocol #12272013.024 PeaceHealth Protocol #551187
November 2018

Contents

PROJECT SUMMARY

As a function of our growing population of older adults, an estimated 3.48 million total knee arthroplasty (TKA) procedures will be performed annually in the U.S. by 2030. Despite the near-universal success of this surgery in mitigating chronic knee pain, TKA is not successful in restoring long-term physical function in older adults, primarily because of quadriceps muscle atrophy, which explains 77% of the strength deficits. Overall, strength and functional mobility in TKA patients is 30-50% below age-matched healthy controls. Functional tasks such as stair-climbing, which is considered a high fall-risk activity, remain a clinical problem for 75% of patients following TKA. Muscle atrophy occurs in both operative and non-operative legs, and is essentially permanent for older patients because their ability to increase muscle mass is significantly impaired. Thus, safe and effective treatments to prevent muscle loss and preserve strength are urgently needed and clinically meaningful, as they have the potential to significantly impact the quality of life for millions of older adults.

The purpose of the proposed clinical research is to determine the effects of essential amino acid (EAA) supplementation on muscle mass, strength, and functional mobility following TKA in older adults. Our hypothesis, based on strong preliminary data, is that twice-daily ingestion of 20 g of EAA (Aim 1) and 23 g of EAA (Aim 2) for one week before through six weeks after TKA will increase basal rates of muscle protein synthesis via inactivation of catabolic signaling, and up-regulation of anabolic and cyto-protective proteins. We further hypothesize that short-term atrophy prevention and accelerated return of functional mobility will lead to longer-term structural and functional adaptations, and improved quality of life in older TKA patients vs. Placebo. The rationale for the proposed research is that effective treatments to prevent muscle atrophy after increasingly common TKA surgery will result in short- and longer-term muscle cell adaptations that boost functional mobility and quality of life. Identifying the mechanisms up-regulated by EAA treatment that preserve muscle volume and mobility will have a major impact on rehabilitation science.

The proposed research will empirically test our hypotheses by accomplishing **two specific aims:** (1) determine if EAA elevates basal rates of muscle protein synthesis by up-regulating anabolic pathways and cyto-protective proteins, and inactivating catabolic pathways in the short term *vs*. Placebo and (2) determine if short-term prevention of atrophy, weakness, and functional mobility leads to positive changes in muscle cell structure and function, and improved quality of life in the longer term *vs*. Placebo. The proposed work is significant because it advances knowledge of the molecular and cellular changes occurring during muscle atrophy (Placebo) and atrophy prevention (EAA) in a clinical setting using a treatment that is broadly applicable, is well tolerated, and can be implemented immediately.

INTRODUCTION AND BACKGROUND

In the coming decades, the demand for primary total knee arthroplasty (TKA) in the U.S. is projected to increase 673% to 3.4 million surgeries performed annually. TKA surgery is primarily performed on older adults and results in significant muscle atrophy in the operative and non-operative thigh (quadriceps and hamstrings), which compromises balance, impairs functional mobility, and dramatically increases the risk of falls and related injuries. For older adults, muscle atrophy occurring during periods of inactivity or following surgery may be more difficult to recover.

Age-associated muscle dysfunction results from reduced muscle mass, strength, and functional capacity (i.e., sarcopenia) (38). Sarcopenia occurs with normal healthy aging and is associated with an elevated risk for falls, physical disability, and loss of independence (105, 106). Estimated healthcare costs due to sarcopenia in the U.S. in 2000 was $18.5 billion (60). Reducing the prevalence of sarcopenia by 10% can save $1.1 billion/year in healthcare costs (60). While sarcopenia progresses at a rate of 1%/year in healthy older males and females, surgeries such as hip and knee replacement, will accelerate sarcopenia (99). The projected increase (nearly 7-fold) in the number of TKA to be performed annually in the U.S. in the coming decades will place a significant burden on our healthcare infrastructure, Medicare and Medicaid. The studies in this research plan are significant because they specifically address a major clinical barrier by mitigating post-operative muscle loss that translates directly into improved strength and functional mobility in older adults. Accelerating functional mobility will help to alleviate a significant economic burden by reducing recovery time, shortening time in clinical care, and halting the acceleration of sarcopenia.

While evidence exists linking EAA to anabolic and catabolic regulation of muscle cell metabolism in healthy subjects (87-89), there is a dearth of experimental data investigating mechanistic adaptations that prevent muscle atrophy and restore function in TKA patients. It is known that EAA stimulates an acute anabolic response in muscle by activating the mTORC1 pathway and increasing fractional rates of protein synthesis (i.e., FSR) (50), and that chronic EAA supplementation elevates fasting rates of muscle protein synthesis in older healthy adults (29). However, studies conducted in healthy populations tell us very little about how muscle cell metabolism is altered following major surgery and if EAA supplementation causes an increase in basal rates of muscle protein synthesis and/or attenuate breakdown. Further, mechanisms linking atrophy prevention with positive functional adaptations in a clinical setting are lacking.

Recent reports demonstrate that changes in fat oxidation in human primary myocytes parallel whole body metabolism of the donor (115). In other words, primary cells isolated from human subjects, which were allowed to expand and become myocytes and subjected to experiments to measure metabolism, revealed that myocyte metabolism were similar to host metabolism. This suggests that normally dormant satellite cells (primary cells) residing in muscle tissue recapitulate the metabolic characteristics of that tissue bed. Further, others have shown that satellite cells from older animals have reduced regenerative capacity as compared to young animals (25), however, when the blood from young mice is mixed with blood of older mice (heterochronic parabiosis) regenerative capacity in older muscles is restored (19, 26). In addition, altering defective signaling pathways in older satellite cells has the ability to normalize their regenerative response to injury (21, 25, 26). While these reports show that the satellite cell microenvironment plays an important role in regeneration a recent report from the Blau lab demonstrate that satellite cells from aged animals have inherent defects in their ability to respond appropriately to muscle tissue injury (27). From these reports we may conclude that satellite cells have metabolic and regenerative characteristics that relate back to the health status and age of the donor. Furthermore, these are important consideration to take into account when interpreting results from

clinical trials evaluating the response(s) of muscle tissue(s), and, more importantly, functional mobility, in older adults.

Recent experiments performed on C2C12 myotubes have demonstrated that following incubation with essential amino acids (EAA) anabolic pathways are upregulated (9), which is similar to the anabolic response measured in muscle cells obtained from healthy young adults after in bolus ingestion (50). Interestingly, we have shown that older adults demonstrate a diminished anabolic response to EAA ingestion relative to younger subjects (41) that is dose dependent (28) and appears to be sensitive to Leucine concentration (63). Interestingly, a recent report from Farup and colleagues (45) showed that subjects supplemented with Whey protein before and after an eccentric contraction protocol demonstrated significantly greater expansion of their satellite cell pool vs. placebo subjects, which is important because Whey supplement has higher concentrations of EAAs and is absorbed quickly relative to intact protein (steak) (95-97). Thus, it appears that EAA supplementation may be acting on muscle tissue by positively influencing satellite cells as well as muscle protein anabolism and/or catabolism. Lastly, recent work from Joshi and colleagues (61) provide methods for assessment of in vitro model of ischemia using myotubes that may be used to isolate pathways that are up and/or downregulated response to ischemia-reperfusion injury. Assimilated, the above information suggest that primary cells isolated from humans of different ages (young vs. old) and varying health status (metabolic) may, under certain experimental conditions, provide valuable insight into donor muscle response(s).

Preventing muscle atrophy and weakness is clinically significant because it leads to immediate and tangible improvements in functional mobility in the short term, which may lead to positive changes in longer-term muscle cell structural and functional adaptations that will improve quality of life for older patients following TKA. Quadriceps weakness inhibits balance (84), reduces functional mobility (20, 82), and increases the risk of falls (83). Muscle atrophy increases the prevalence of physical disability (103) and accelerates the progression of sarcopenia (99). Tests of functional mobility consistently demonstrate marginal improvements following TKA surgery (47, 92, 123). Functional tasks such as stair-climbing, which is considered a high fall-risk activity, remains a clinical problem for 75% of patients following TKA (92). A recent report suggests that early post-operative function may predict the speed of functional recovery and the overall success of rehabilitation (64). Evidence suggests that acute weakness in the non-operative limb following TKA is related to poorer functional outcomes in the long-term (126), and maintaining greater muscle volume in the operative leg is essential to maximize muscle strength (79, 80) and attenuate the acceleration of sarcopenia (99). Studies show that, after a peak in functional recovery at 6 months, there is an acceleration of functional decline due to quadriceps weakness that outpaces normal 'aging' that is not attributable to lack of range of motion or pain (81, 82, 98, 125). Reports suggest a link between mitochondrial dysfunction and aging (2), which is exacerbated by inactivity (5). Preserving muscle volume and cellular function is essential to improve strength and accelerate functional mobility, which we hypothesize will lead to positive structural and functional adaptations in muscle cells at 6 months post-TKA (79, 80).

Preliminary Studies.

Biochemistry. Recent studies in the Dreyer Laboratory have generated exciting preliminary results showing essential amino acid (EAA) supplementation attenuates quadriceps atrophy and accelerates the return of functional mobility following TKA (37). For patients on EAA, quadriceps atrophy was only -6% & -3% in the operative and non-operative quadriceps, respectively, six weeks after TKA but -18% & -10%, respectively, in patients on Placebo (a 3-fold difference). Of clinical relevance, these patients on EAA were able to maintain strength and demonstrated an accelerated return of functional mobility vs. Placebo, 6 wk post-TKA. At the cellular level, our preliminary results show that EAA supplementation

down-regulated catabolic signaling (FoxO3a) and up-regulated amino acid transporter (SLC36A2), regulated in development and DNA damage response (REDD1) and activating transcription factor 4 (ATF4) expression vs. Placebo. While studies by others have linked these molecules with the insulin like growth factor (IGF-I) and vascular endothelial growth factor (VEGF) (i.e., angiogenesis) pathways, whether they are directly stimulated by EAA supplementation in a clinical population remains unknown. We interpret these positive changes at the cellular level to be responsible for the reduction in muscle loss and, preservation of strength, and explain the acceleration of the return of functional mobility.

Our preliminary fractional synthesis data (FSR) show that older adults respond appropriately to acute ingestion of 20 g of EAA (i.e., similar response to young healthy controls). We also have preliminary FSR data showing that basal rates of muscle protein synthesis are similar between TKA patients (0.0563 ± 0.008%/hour) and older healthy control subjects (0.0566 ± 0.008%/hour). What is not known is what effect TKA has on muscle protein synthesis and breakdown in response to chronic EAA supplementation (for 1 week prior to, and for 6 weeks post-TKA). We have extensive experience with stable isotope tracer methods to determine fractional synthesis rates (FSR), a direct measure of muscle protein synthesis (31-35, 39-41, 50, 113, 114). However, in order to determine mechanisms, and link cellular changes in anabolic and catabolic pathways with atrophy (Placebo) and atrophy prevention (EAA) we must also quantify muscle protein breakdown.

Our preliminary results show that EAA phosphorylates (inactivates) a primary regulator of muscle protein catabolism (FoxO3a). As well, EAA supplementation stimulated the expression of the anabolic amino acid transporter, which has gained attention due to its ability to act as both a transporter and signaling protein linked to the IGF-I signaling pathway (54, 102). Interestingly, we also measured an up-regulation of the transcription factor ATF4, which has been shown to stimulate the expression of amino acid transporters and promote protein anabolism (76). In vivo and in vitro experiments have shown that IGF-I treatment increased REDD1 expression in muscle (49), and recent studies have shown that REDD1 confers cyto-protective effects by mitigating cell stress (22, 120). REDD1 is upregulated by ATF4 (49, 76, 122). ATF4 is increased in the presence of insulin (4) and IGF-I (49), and plays an important role in mediating the effects of VEGF on cell growth and survival (3, 75, 93). Thus, ATF4 may be necessary for amino acid and protein anabolism (4). We have preliminary data showing that REDD1 expression is elevated with EAA. This is important because our preliminary results support mechanisms of action by which EAA up-regulate amino acid transporters via ATF4 that have implications for muscle protein metabolism (76). We interpret these results as supporting a mechanistic role in atrophy prevention.

Physiology. Immobilization following orthopedic surgery accelerates sarcopenia (99). For example, 1%/year = normal sarcopenia progression, but in our TKA patients, sarcopenia was accelerated by +18 and +10 years, in the operative and non-operative leg, respectively. However, in patients randomized to EAA, progression was only +6 and +3 years, respectively. Thus, EAA have the potential to mitigate early onset of sarcopenia. Preventing muscle atrophy bilaterally is clinically important, as early post-operative quadriceps weakness in the non-operative leg is directly linked to poorer long-term functional outcomes (126).

Quadriceps weakness results from both muscle atrophy and neural activation deficits (80, 107). In the longer term however, strength deficits are due to persistent muscle atrophy, explaining 77% of the quadriceps strength deficits 1 year post-TKA (80). Our preliminary data show that EAA supplementation reduces strength loss and accelerates the return of functional mobility. Persistent quadriceps atrophy and weakness have profound effects on functional mobility and activities of daily living (13, 107, 119). It

is well known that quadriceps mass and strength are strong predictors of functional mobility (i.e., timed up-and-go, stair-climb down [descent] ability and ambulation).
We also have compelling preliminary data showing that Type II muscle cell cross-sectional area (CSA) is reduced (atrophied) compared to controls following TKA. We expect to show that CSA increase (normalize to that of the non-operative side) in the EAA group. As well, we have preliminary data from isolated muscle fiber bundle analysis of oxygen consumption (mitochondria respiration) from muscle cells from TKA subjects have lower (-61%) basal mitochondrial function (respiration) vs. age- and sex-matched controls.

Contribution to Scientific Knowledge.

Muscle atrophy occurs in cancer cachexia (12), chronic heart failure (8), chronic obstructive pulmonary disease (70), HIV-AIDS (121, 124), renal failure (66), rheumatoid arthritis (71, 77), osteoarthritis (116), burn injury (18), following total hip and knee arthroplasty (58), ACL reconstruction (57), and during bed rest (94). Despite its widespread impact in clinical settings little progress has been made in devising ways to prevent muscle atrophy. As such, our R01 proposal is significant because it has broad clinical appeal as a high yield therapy to attenuate atrophy under a variety of clinical conditions (EAA are well tolerated, easily digested, and immediately available).

The significance of this Research Plan also centers on our use of TKA as a model system for the study of muscle atrophy (Placebo) and atrophy prevention (EAA), which are due to an imbalance between anabolic and catabolic alterations at the molecular and cellular level. *Our study will advance knowledge of the molecular and functional changes that are occurring during muscle atrophy and prevention in older males and females, and will identify additional novel targets for therapeutic interventions.*

Our study is innovative for several reasons. Foremost is our translational approach which will allow us to identify novel molecular regulators of muscle atrophy (Placebo) and atrophy prevention (EAA). Using isotopically labeled tracer methodologies we will quantify muscle protein synthesis and breakdown, and link those results with changes in muscle volume, strength and functional mobility. We will also determine the degree to which short-term preservation of muscle volume, strength and function translate into longer-term structural and functional (mitochondrial respiratory) adaptations at the cellular level. Our plan to explore potential sex differences in the age-related changes in muscle protein synthesis and breakdown following TKA and the mechanistic effects of EAA on muscle metabolism, atrophy, and mobility is exciting. Finally, this research plan brings together a clinical and translational research team to investigate the biochemistry and physiology of muscle atrophy and incorporates functional measures of mobility and quality of life in a unique and meaningful way.

The primary innovation is our integration of cellular and molecular methods with measures of muscle protein synthesis and breakdown, volume, and functional mobility in order to better understand how muscle atrophy can be prevented in a clinical setting(s). Our protocol will be freely available to clinicians, therapists, and researchers interested in mitigating muscle atrophy, which may profoundly affect the medical/rehabilitation field as the use of EAA in atrophy prevention spreads and gains momentum.

Hypothesis.

The working hypothesis, based on strong preliminary results, is that twice-daily ingestion of 20 g of EAA for 1 week before and 6 weeks after TKA, will increase basal rates of muscle protein synthesis via inactivation of catabolic signaling (FoxO3a), and up-regulation of anabolic (SLC36A2, IGF-I & VEGF) and cyto-protective proteins (ATF4 & REDD1). We further propose that short-term atrophy prevention and accelerated return of functional mobility will translate into long-term (6 months post-TKA) structural (cross-sectional area, CSA) and functional (mitochondrial respiration) adaptations, leading to improved quality of life in TKA patients vs. Placebo in older men and women.

SPECIFIC AIMS

To test our hypothesis our *Research Plan* proposes scientifically rigorous methods designed to achieve the following specific aims:

- **Specific Aim #1 (Biochemistry): Determine if EAA elevates basal rates of muscle protein synthesis by up-regulating anabolic pathways & cyto-protective proteins, and inactivating catabolic pathways in the short-term vs. Placebo.**

  Identifying molecular pathways that are up-regulated by EAA are needed in order to uncover potential mechanisms of action. Successful completion of the proposed research will determine the degree to which EAA elevate basal rates of muscle protein synthesis and the extent to which these changes coincide with increases in anabolic (SLC36A2, IGF-I, & VEGF) and cyto-protective (ATF4 & REDD1) pathways. As well, completion of Aim 1 will allow us to determine changes in muscle protein breakdown in parallel with changes in catabolic (FoxO3a) proteins (Fig. 3). We expect these findings will provide mechanistic data on the effects of EAA on muscle cell metabolism and signaling that will help explain how muscle atrophy is prevented and functional mobility accelerated following TKA.

  To accomplish Specific Aim #1, subjects will be enrolled pre-operatively and asked to participate in a number of study-related activities designed to isolate the effect of EAA supplementation on muscle protein synthesis. Subjects will consume “heavy water” to aid in quantifying chronic muscle protein synthesis rates while consuming the study EAA supplement. Study specific blood draws, muscle imaging (via MRI) and muscle biopsies will be assessed at regular intervals. Patients will capture accelerometer data and complete three-day food diaries, as well as patient reported outcome measures designed to capture health-related quality of life, knee-specific functioning, pain and the degree to which the patient has an ability engage in total joint pre- and post-operative care. Finally, functional testing will capture patient strength and functional capabilities pre- and post-operatively.

The proposed research design will answer the following questions:

1.1 Does EAA elevate or alter levels of muscle protein synthesis vs. Placebo?

1.2 Does EAA decrease or alter levels of muscle protein breakdown vs. Placebo?

1.3 Will EAA inactivate catabolic (FoxO3a) and up-regulate anabolic (SLC36A2, IGF-I, ATF4 and REDD1) pathways vs. Placebo?

1.4 Does inhibition of angiogenic factors (IFG-1 & VEGF) inhibit a pro-angiogenic response?

1.5 Does EAA influence markers of inflammation vs. Placebo?

1.6 Does EAA influence markers of mitochondria function vs. Placebo?

1.7 Does statin use influence cell respiration over time?

1.8 Does EAA affect primary cell derived myocyte function over time?

- **Specific Aim #2 (Physiology): Determine if short-term prevention of atrophy, weakness, and functional mobility leads to positive changes in muscle cell structure and function, and quality of life in the long term (6 months post-TKA) vs. Placebo.**
Successful completion of Aim 2 will identify a role for EAA supplementation in clinical settings using a mechanistic approach. Our approach is based on strong preliminary clinical data from older men and women following TKA demonstrating that EAA supplementation for 1 week prior to, and for 2 week post-TKA was effective in preventing significant muscle atrophy (37). However, our preliminary data suggest that in the EAA group, atrophy at 2 weeks post-TKA was half that at 6 weeks in the operative quadriceps and hamstrings. This potential 'catch-up' muscle loss was not observed in the non-operative leg within the EAA group but was identical to the change from 2 weeks to 6 weeks in the operative leg of the Placebo group. We interpret these data to support the need to supplement for 6 weeks post-TKA in order to maximally prevent bilateral muscle atrophy.

  The proposed research design will answer the following questions:

  2.1 Does EAA prevent short-term (7 wk post-TKA) bilateral muscle atrophy, preserve quadriceps strength, and accelerate the return of functional mobility vs. Placebo?

  2.2 Are there short-term sex differences on the above outcome measures?

  2.3 Will EAA increase long-term (6 mo post-TKA) quadriceps functional mobility vs. Placebo?

  2.4 Does EAA improve longer-term (6 mo post-TKA) functional mobility and measures of quality of life vs. Placebo?

  2.5 Are there long-term sex differences in functional mobility or measures of quality of life?

  2. 6 Does inhibition of angiogenic factors (IFG-1 & VEGF) inhibit a pro-angiogenic response?

  2.7 Does EAA influence markers of inflammation vs. Placebo?

  2.8 Does EAA influence markers of mitochondrial function vs. Placebo?

  2.9 Does statin use influence cell respiration over time?

  2.10 Does EAA affect primary cell derived myocyte function over time?

Methods, Materials and Analysis

Study Design.

Prospective, randomized, blinded, controlled, two-arm, parallel clinical trial design with 1:1 allocation ratio to EAA versus Placebo. Staged enrollment is planned; we will complete Aim #1 before enrolling into Aim #2.

Investigational Supplement.

This trial is being conducted under IND#121601, with mandates to report results from Aim #1 to the Food and Drug Administration (FDA). The investigational supplement (or Placebo) will be ingested twice-daily, at 10:00a and 2:00p for one week prior to TKA surgery.

- Aim 1 -Supplement will be compounded by Northwest Compounders, and ordered per standard of care for pharmaceutical ordering (fax or phone call) and dispensing.

- Aim 2 – Supplement will be supplied by MEND Nutrition, Inc. Supplement will come individual containers shipped directly to the Dreyer Lab. Containers will be distributed to patients by study coordinators. Patient will measure two (2) scoops per dose (twice daily). Scoop provided in each container.

Aim 1 Supplement will be suspended on the day of surgery and resume on post-operative day #1. On post-operative days where subject participates in physical therapy, supplementation will occur within one hour of treatment (20 g of EAA or Placebo). The EAA supplement or Placebo composition is described in Table 1 below. Our preliminary study suggest the EAA supplementation is safe and no subjects withdrew due to problems with supplement ingestion (37).

Aim 2 Supplement will be suspended on the day of surgery and resume on post-operative day #1. On post-operative days where subject participates in physical therapy, supplementation will occur within one hour of treatment (23.42 g of EAA or Placebo). The EAA supplement or Placebo composition provided by MEND Nutrition, Inc. is described in Table 2 below. Our preliminary study suggest the EAA supplementation is safe and no subjects withdrew due to problems with supplement ingestion (37). Supplement composition has changed due to new manufacturer/supplier of the supplement (MEND). To our knowledge there are no scientific or risk implications related to the change in proportions.

Recent results from the Phillips group (128) demonstrated that free-living older males taking 5 grams of Leucine with each meal (3x/day for 3 days), placed on either a low- (RDA; 0.8g protein/kg/day) or high- (1.2g protein/kg/day) protein diet, had elevated basal and post-exercise myofibrillar protein synthesis vs. the same individuals on the same diets (low or high) without supplemental Leucine. Subjects on low-protein and high-protein diets supplemented with 5g of Leucine were not different from one another in this study. The finding has direct implications for our patients who eat less after surgery (37). Data for the Phillips paper were obtained by using D_20 and reflect the rates of myofibrillar synthesis over 3 days in individuals who consumed their normal meals and performed their usual level of activity. This suggests to us that using doses of Leucine closer to 15g/day may have a more profound effect in offsetting muscle autophagy, which can be stimulated by mTORC1, which is activated by Leucine (129).

This will bring us close (13.6g/day) to the 15g/day used by the Phillips group. These more current findings justify modifying our protocol in order to maximize our ability to positively influence patient outcomes.

Participant compliance will be assessed by two primary mechanisms: (1) log-book inspection which the subject will record each supplement ingestion time and (2) empty vial collection. Supplements will be randomly tested for quality control using HPLC to ensure no mixing of samples between groups.

Table 1: Composition of investigational supplement (EAA) and Placebo – Aim 1.

Composition	%	g
Essential Amino Acid		
Histidine	11	2.2
Isoleucine	10	2.0
Leucine	18	3.6
Lysine	16	3.2
Methionine	3	0.6
Phenylalanine	16	3.2
Threonine	14	2.8
Valine	12	2.4
Placebo		
Alanine	100	20

Table 2: Composition of investigational supplement (EAA) and Placebo – Aim 2

Composition	%	g
Essential Amino Acid		
Histidine	5	1.28
Isoleucine	8	1.8
Leucine	32	7.4
Lysine	15	3.6
Methionine	8	1.76
Phenylalanine	13	3.1
Threonine	8	1.9
Valine	9	2.08
Tryptophan	2	0.5
Placebo		
Alanine	100	23.42

Study Aim #1 (Biochemistry).

Aim #1: Determine if EAA elevates basal rates of muscle protein synthesis by up-regulating anabolic pathways & cyto-protective proteins, and inactivating catabolic pathways in the short-term vs. Placebo.

Randomization Assignments. Aim #1 will consist of 2 groups (N=20/group through protocol completion; EAA vs. Placebo) that are identical in protocol design except for metabolic studies. Metabolic studies will partition the EAA group into 2 (post-TKA bilateral biopsy at 1 or 2 weeks after

surgery) and Placebo group into 2 (post-TKA bilateral biopsy at 1 or 2 weeks after surgery). This will allow us to collapse data by group treatment group; questionnaire, activity, food-log, strength & activation, muscle volume and functional data (this equals N of 20 through protocol completion for EAA and N of 20 for Placebo through protocol completion; 20/group, enough for sex comparison), while enabling us to quantify changes over time in metabolism (D_20) and at discrete time points after surgery (1 and 2 weeks post) between legs when 80% or more of muscle atrophy is occurring (37).

Primary Biochemical Outcome Measures.

To answer our study questions will be answered using an in vivo experimental design**. The procedure schedule can be referenced in Figure 1 (page 17, following a description of all study-related procedures and measures for Aim #1).**

Deuterium Oxide Ingestion Followed by Muscle Biopsies. Deuterium oxide (D_2O, i.e., heavy water) will be used to quantify chronic (days) muscle protein synthesis rates. D_20 will be ingested (50 ml) three times per day for 7 days leading up to TKA. During TKA surgery, a single biopsy will be obtained in the OR from each leg. Beginning on POD#1 and continuing through week 1 or week 2, subjects will continue ingesting 50 ml of D2O twice daily. Bilateral biopsies obtained from each leg at 1 ***or*** 2 weeks post-TKA will be analyzed to determine muscle protein synthesis rates. All subjects will have a total of two biopsies from each leg, in a fasting state. This will allow us to determine the independent effects of supplementation on rates of muscle protein synthesis and breakdown ($^2H_20$) during the acute and sub-acute period.

Measures include structural (CSA of cell and extracellular matrix) and functional (mitochondrial respiration & inflammatory cell infiltration) determination. Muscle cell structure (CSA) and function (mitochondrial respiration) will be determined based on percent change from baseline and made relative to the non-operative leg (internal control). From muscle biopsies we will also isolate primary muscle cells (i.e., satellite cells).

To perform the biopsies, a small incision about the size of this dash “---" approximately at mid-thigh, through which a needle about the size of this letter "O" will be advanced into the muscle and fat. A piece of tissue will then be removed. This will be done on each leg.

The postoperative biopsies will be performed by the Principal Investigator (Dreyer). Dr. Dreyer has performed over 500 biopsies and he has performed over 200 tracer studies. The biopsies will be performed in his lab at the Center for Medical Education and Research, or at the Slocum Center, in the morning in a fasted state. This procedure involves the taking of a small piece of tissue from the leg, containing both fat and muscle. The skin is cleaned and made sterile, and the skin and tissue below are injected with local anesthetic, lidocaine (numbing medicine), to eliminate pain. Afterwards, the skin will be closed with a single stitch (Aim 1 only). The stitch will be removed at the lab in about 7 days (plus or minus one day to accommodate participant schedule).

During the surgery, the surgeon or one of his assistants will perform the biopsies. The patient will still be under anesthesia. The intraoperative biopsies will not have a stitch that requires removal. The site will be covered with a sterile dressing.

Primary cell isolation. Primary cells isolation studies will be performed using standard methods that exist in our lab in order to conduct experiments on muscle cells that could not be perform on subjects

Muscle cells may be manipulated in a laboratory environment, and much learned from the manipulation, in ways that are not possible in vivo. For example, we can block metabolic processes from happening and evaluate the impact on muscle tissue. This approach will have the added benefit of increasing the amount of muscle tissue we have to work with. However, this approach is not intended to develop perpetual cell lines. The myocytes are in cell culture, they have a finite life span and will expire within 2 to 4 weeks. After the experiments on cells are completed they will be dead already or destroyed.

Muscle Morphology. Morphology will be determined from tissue obtained using immunohistochemical techniques to examine differences in muscle fiber cross sectional area (CSA), and fiber type. CSA will be determined using standard methods (7). Tissue sections will be cut at 10µm thickness in a Leica CM 1850 UV cryostat maintained at -22ºC. Each tissue section will be mounted on Fisherbrand Superfrost®/Plus microscope slides (Fisher Scientific, USA). Digital images will be captured on a Leica DM4000 Epi-Fluoroscent microscope and analyzed using MetaMorph Premier Software.

Westerns. Specific cell signaling pathways probed will be stress response and anabolic and catabolic pathways. The PI has extensive laboratory experience and training in these methods, techniques and application of immunoblotting techniques (2, 3, 5-7, 10, 12). The phosphorylation status of each protein will be expressed relative to total protein after each is individually expressed relative to GAPDH or α-Tubulin. Anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody will be purchased from Amersham Bioscience. Western blot signals will be analyzed with a BioRad XRS Chemiluminescence imaging system, which detects ECL signals over 5 orders of magnitude, and its sensitivity range far exceeds that of conventional densitometry.

Gene Expression: RNA will be extracted from 15-20 mg of muscle tissue according to protocol (Molecular Research Center, Inc, Cincinnati, OH). High quality RNA will be determined using lab-on-chip microfluidic technology automated nucleic acid electrophoresis and analysis. Total RNA will be reverse transcribed into cDNA using the iScript cDNA Synthesis Kit (Bio Rad, Hercules, CA) on a C1000 Thermal Cycler (Bio Rad, Hercules, CA). Determination of relative mRNA expression will be performed using a CFX96 Real-Time System coupled to a C1000 Thermal Cycler (Bio Rad, Hercules, CA) and Genomics Core facility NextSeq 500 at the University of Oregon.

Mitochondrial respiration. Oxidative phosphorylation rates in response to 50µM ADP will be determined in saponin permeabilized vastus lateralis biopsies (5-10 mg) using the two-channel high resolution respirometer (Oroboros Oxygraph; Innsbruck, Austria) with the saturating concentrations of the following substrate combinations [in mM]: pyruvate [5] + malate [5], palmitoylcarnitine [0.04] + malate [5], succinate [10] + 100 nM rotenone, or malate [5] + glutamate [10]. Respiration studies were performed at an initial oxygen concentration of 300 µM at 37C in MiR06 respiration buffer containing [in mM] EGTA [0.5], $MgCl_2 \cdot 6\ H_2O$ [3], K-lactobionate [60], taurine [20], KH_2PO_4 [10], HEPES [20], sucrose [110], 1g/L BSA, 280U/ml catalase, pH 7.4 [with KOH].

Mitochondrial dysfunction may introduce sample bias as well as negatively impact short and longer-term function. To account for this, we propose to measure mitochondrial respiration using the Oroboros O2k respirometer, and normalize results statistically and use the non-operative leg as internal control.

Secondary Outcome Measures. Study Aim #1 will allow the team to collect preliminary functional outcome data to better inform the long-term study (Aim #2). The secondary outcome measures will be quantified using the following study activities and procedures:

- **Muscle volume.** The MRI unit is located at the Lewis Center for Neuroimaging (LCNI) on the University of Oregon campus. Should the MRI unit at LCNI be unavailable within study-specific assessment points (i.e. scheduling or mechanical issues), the MRI at the Slocum Center for Orthopedics will be utilized. In addition, patients in partnership with the study team, may elect to have all scans performed at the Slocum Center.

  All scans will be performed prior to strength & functional testing and a minimum of 24 hr post-therapy. T1 & T2 weighted MR images will be used to quantify quadriceps volume vs. edema. MRI is safe for TKA patients (implants are non-ferrous) and is ideally suited for muscle volume quantitation. Capture protocols will be stored on the unit to ensure accurate image collection over the course of the study. MRIs will not be performed in a fasting state.

  MRIs performed at the Slocum Center will follow the standard operating procedures of Slocum MRI as per standard of care. Slocum MRI will carry out their own screening processes at the time of the scan. Research staff will alert MRI staff if the patient has any contraindicated implanted devices prior to the scan. Slocum MRI will follow their standard operating procedures to ensure MRI safety.

  Because MRIs at the LCNI are strictly used for research purposes, study participants will complete the IRB-approved LCNI screening form in advance of their MRI procedure(s). Based on the screening form results if LCNI requires more information to comfortably scan our patients, LCNI will call the study coordinators. Information that may be requested by LCNI includes any of the following (more than one possible):
  1. Confirmation of MRI scan with implant in question has occurred previously
  2. Blinded operative notes of the implant in question
  3. Advance conversation with the patient at LCNI
  4. Communication /confirmation/conference with surgeon
  5. Identification of implant type

  If the patient requires any #2-5 above, a separate HIPAA authorization to communicate with LCNI will be completed. For patients who have pre-operative and post-operative research scans at the Slocum Center, data will be stored per standard of care; however, images will be transported through a secure VPN connection for analysis at the Dreyer Lab.

  It is important patient's have pre-operative and post-operative scans on the same MRI machine. The study team will work with the patients and his/her care team to determine the MRI location to best serve the patient's needs.

- **Edema and fluid accumulation in the surgical versus nonsurgical thigh.** From the biopsies, muscle samples will be weighed wet and then dried and weighed again. The difference between the two weights will be used to estimate fluid accumulation in the muscle tissue. Biopsies will be performed in a fasting state.

- **Function.** Standardized grip strength, standing balance, chair stand test, timed up-and-go (TUG), timed stair ascent and timed stair descent, 4 meter walk test and the 6 minute walk will be assessed. Grip strength will determine changes in each hand grip over time. Standing balance will measure changes over time in balance. The chair stand test measures the time it takes for

the subject to stand and sit five times. TUG will measure the time (seconds) needed to stand from a chair, walk 3 meters, turn around, walk back and be seated again. Timed stair ascent (10 steps) and descent will be recorded. The 4 meter walk will tell us how long it takes you to walk a short distance (4 meters) while the 6 minute walk will measure the distance you can walk over a longer distance (total distance in 6 minutes). We have experience with these functional measures. Each functional mobility measure will be performed on a single standardized chair (TUG) and stairs. Functional assessments will not be performed in a fasting state. Functional measurements may be performed at either the Dreyer Lab on the University of Oregon campus or the Slocum Center for Orthopedics & Sports Medicine.

- **Strength & central activation deficit.** Co-Investigator Dr. Anita Christie, PhD, will contribute her expertise to quantify pre- and post-operative changes in strength and quadriceps activation (24). Strength will be quantified using a Biodex +/- tests for central activation deficits (CAD). CAD contributes to early weakness following TKA, and Dr. Christie will quantify this progression between groups over time using previously published methods will be followed (23, 44, 62, 65, 67, 112). Voluntary activation of the knee extensor muscles will be assessed through the painless method of supramaximal magnetic stimulation of the femoral nerve superimposed on the MVC (90). Central activation of the knee extensors will be calculated from the resulting force as follows: (MVC/(MVC + Stimulation)) x 100 (65). Nerve function will be determined using the latency of the M-wave in response to supramaximal magnetic stimulation of the femoral nerve (23, 44). Additionally, pre-post surgery measures of the area of the negative peak of the M-wave will be used to estimate motor neuron loss (67). Strength and central activation deficits will not be performed in a fasting state. Central activation is measured on the University of Oregon campus or at the Slocum Center.

- **Patient-reported outcome measures**. Patients will be asked to complete questionnaires assessing self-reported quality of life, knee-specific functioning and pain.

    1. ***VR-12*** - Consists of select items from the eight concepts of health in the SF-12, summarized in a physical component summary and a mental component summary. Useful in understanding health-related quality of life, and differences in disease burden. Administered at Slocum per standard of care via iPads.

    2. ***KOOS*** - Well-established standardized knee-specific functional outcomes tool. Used frequently in the literature to report degree of functional mobility and knee-related disability. WOMAC scores may be calculated from KOOS. Reviewers may expect KOOS/WOMAC data collection use in orthopedic clinical trials. Administered on paper for research purposes.

    3. ***Oxford Knee*** - Shorter knee-specific functional outcomes tool. Suggested to have superior validity, reliability and responsiveness compared to older knee-specific outcome tools. Well established for use in Europe, and has gained significant traction in the United States over the past few years. Reviewers may expect Oxford data collection use in orthopedic clinical trials. Administered at Slocum per standard of care via iPads.

    4. ***Visual Analogue Pain Scale (VAS)*** - Measures self-reported pain, which is associated with functional outcomes in orthopedic clinical trials. Administered at Slocum per standard of care via iPads.

Covariate Measures.

There are a number of study-related activities employed to allow the study statisticians to isolate the effect of EAA supplementation on primary and secondary outcome measures (described above).

- **Accelerometer.** All subjects will wear an ActiGraph (Pensacola, FL) GT3X accelerometer at various points throughout the study, including during inpatient and outpatient PT (see *Study Timeline*). Participants will be instructed to wear the device at all times except when showering, bathing, swimming, or sleeping. Accelerometry data will be downloaded to a computer using ActiLife v.6 software. Total daily energy expenditure (TDEE; kcal/day) will be estimated, and physical activity levels (PALs; TDEE/basal metabolic rate [BMR]) calculated to adjust for body size. Activity energy expenditure (AEE; kcal/day), calculated as TDEE-BMR, will be used to describe the caloric costs of physical activity. In addition, time spent in standard categories of activity (sedentary, moderate, vigorous) will be assessed. Our study coordinator will exchange accelerometers every week, which we have determined from our preliminary studies to maximize battery life and capture all activity data.

- **3-day food log.** All subjects will receive a thorough explanation of, and examples of, how to complete the 3-day food log. In addition, food intake will be captured by study coordinators and/or hospital staff while the patient is admitted. Our study coordinator will interact with subjects in person and by phone to monitor compliance. In addition to monitoring food log compliance, the study coordinator will collect supplement containers to ensure compliance between groups. Carbohydrate, fat, and protein intake will be incorporated into our statistical analysis. A copy of the 3-day food log can be reviewed in Appendix 2.

- **Patient-Reported Outcome Measures.** Additional questionnaires will be collected for conditions that may impact our primary and secondary outcome measures.
    - ***PHQ-9*** **-** Depression has been associated with orthopedic outcomes for total knee arthroplasty procedures. The presence and severity of depression may confound study results if not measured. Administered on paper for research purposes.
    - ***Life Events*** **-** Similar to the rationale for depression, significant life events may impact overall health status, thereby possibly confounding primary study endpoints. Capturing significant life events will allow for investigators to control for externalities that may otherwise go unmeasured in total knee outcome studies. Administered on paper for research purposes.
    - ***Patient Activation Measure (PAM)*** - Measures the degree to which patients have the confidence, knowledge and ability to manage health conditions, health needs and medical care. Patient Activation level has been associated with cost, utilization and health outcomes. Administered at Slocum under a separate research protocol (data to be used for both studies).

- **Patient Demographics, Medical History, Limited Social History, Medications.** Please refer to Appendix 1 and the Data Specifications Table to review covariate measures collected for this protocol.

Blood Draws.

Table 2 describes the blood tests required to achieve study aims or, as requested by the Food and Drug Administration (FDA) under the IND requirements. Each subject will have daily AM fasting blood draws (18 ml). Standard of care includes blood draws at the pre-operative visit with the anesthesiologist and daily am blood (7 ml) draws while in the hospital. Should the pre-operative visit with anesthesia occur prior to study enrollment, the patient will be asked to have research-only labs drawn prior to initiating supplementation (Aim 1). Aim 2 preoperative blood draw will not overlap with standard of care. Some patients may require a blood draw on the day of surgery under standard of care (and required for research purposes), or additional labs (e.g. AM and PM) while in the hospital; however, the typical patient will have between four and five blood draws during the TKA course of treatment. At each standard of care blood draw, one or two tubes of blood are collected. For research purposes, to satisfy the FDA and research requests, four additional tubes of blood will be drawn (tests for: homocysteine, C - reactive protein, standard lipid panel, and ketone assay). While the subject is in the hospital, they will only have the additional tubes of blood drawn on the day of surgery and post op day two. In addition, we will collect two extra tube of blood for analyzing at the Dreyer Lab at each blood draw. One tube is a serum collection tube and the other a plasma collection tube. Both types of blood product are required to conduct the analysis described in the study procedures.) In total, at each study time-point (pre-admit, day of surgery, post op day two, and two and six weeks post-TKA), seven or eight tubes of blood are collected (routine care plus study bloods).

For pre-admission blood work, the surgeon requires additional blood work to assess for blood clotting (PT/INR) and type and screen (i.e. blood type) per standard of care. Pre-admission blood work will not be performed in a fasting state. No ketone assay is collected at pre-admission, resulting in two tubes of blood per standard of care, plus five tubes of research-specific blood work.

Through hospital discharge, labs collected overlap with standard of care time-points. Inpatient blood draws will be performed in a fasting state. There are two additional blood draws required that are for research purposes only: two weeks post-TKA (Aim 1) and six weeks post-TKA, which will also be performed in a fasting state. All eight tubes of blood collected at two weeks, and all seven tubes of blood collected at six weeks post-TKA are for research purposes only.

Each blood tube collected is between 2.7mL and 7.0mL, depending on the blood test requirements. The amount of blood collected at each blood draw is between 28mL and 39mL (high estimate). As reference, when a person donates blood, 500-550mL of blood are donated. Even with seven tubes of blood, the amount collected at each draw is approximately 8% of the amount required at a typical blood donation.

Research blood will be kept on ice on the floor/unit for research staff pickup.

A copy of the laboratory results collected for study-related procedures will be provided to the patient's primary care provider. Transmission of results will occur under standard HIPAA protocols for clinic to clinic sharing of information (e.g. fax). The patient's primary care provider may elect to follow-up on results to assure high quality care and care management. Patients will authorize lab result sharing through the study-specific HIPAA Authorization for Research.

Table 2. Study blood draws identifying timeline, type of blood test and requirements satisfied.

Laboratory Test	(- 6 wk to -1 wk less 1 day))	Day of Surgery	Inpatient	2 weeks post-op$^{\beta}$ (± 2 days)	6 weeks post-op (± 2 days)	As needed for:
Comprehensive Metabolic Panel	X*	X	X	X	X	FDA
CBC, no differential	X*	X*	X*	X	X	Research
Homocysteine	X	X	X	X	X	FDA
C-Reactive Protein	X	X	X	X	X	FDA
Standard Lipid Panel	X	X	X	X	X	Research
KetoneAssay		X	X	X		Research
7 ml K3 EDTA tube (extra blood)**	X	X	X	X	X	Research
5 mL SST tube (extra blood)**	X	X	X	X	X	Research

**Overlaps with routine standard of care.*
***Collected to achieve primary biochemistry outcome measures.*
β Aim 1 time point only

Physical Therapy (PT).

Inpatient PT will be standardized for all patients as follows: Post-op day zero (POD#0). Subject will have same-day evaluation if the patient is stable and is transferred to the PT floor by 3:30 pm. POD#1: Patient will have an individual treatment between 9:00 am and 11:00 am and group therapy session at 1:30 pm. POD#2: Patient will have group therapy at 9:30 am and 1:30 pm, and individual treatment. POD#3: 9:30 am exercise class followed by discharge if medically cleared. Supplement will be ingested within 1 hr after morning and afternoon therapy to maximize anabolic effect at each time point (32).

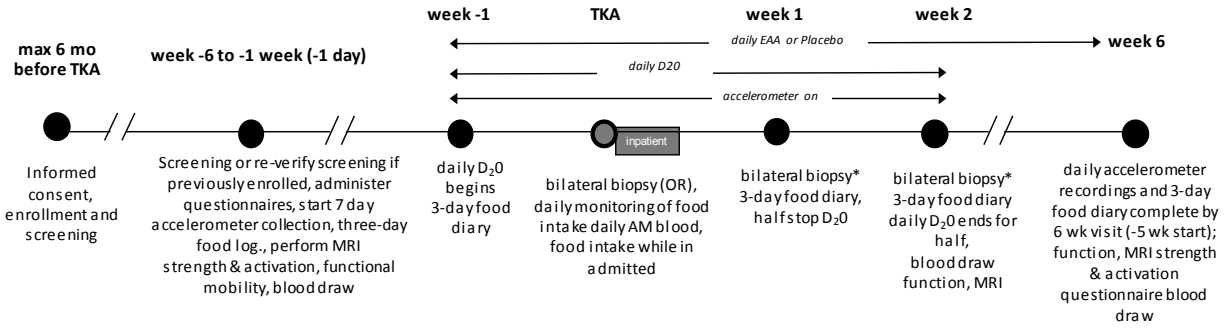
Ms. Embree, PT, and Dr. Dreyer will facilitate standardization of outpatient PT at all locations; however, each patient is individually treated and subjects in EAA may respond/advance through therapy faster, potentially requiring fewer treatments, which would be a positive outcome. This will be accounted for statistically. All subjects will have 14 outpatient therapy visits as follows: Post-op days #5 and #7 for initial evaluation and first treatment. Thereafter PT will routinely see patients 3 times/week for 2 weeks and then 2 times/week for 4 weeks. Physical therapists will be asked to complete a standardized assessment form to document the patient's range of motion, leg circumference and types of exercises performed. The standardized PT data collection tool will be completed by the patient's physical therapist at the first outpatient PT appointment and on the last day treated each week, beginning the week following discharge and ending at post-operative week #5.

For Aim #2, the count of PT visits will be captured as well of date of discharge from formal therapy; however, community therapists will not be asked to complete a standardized assessment. Range of motion at standard of care six week and three month post-op appointment with their surgeon will be obtained.

Aim #1 Study Activities Timeline.

Patients may be enrolled up to six months prior to surgery, and remain on the protocol until 6 weeks ± 2 days post-TKA (up to 7.5 months of total enrollment). Figure 1 (next page) describes the schedule of study-related events, including evaluable time windows, under Study Aim #1.

Figure 1. Overview of Aim #1 timeline and all study activities, measures and procedures.



Schedule of Events								
Time	**-6 mo max**	**-6 wk to -1 wk (-1 day)[B]**	**-1 wk (± 0 day)**	**TKA (Day 0)**	**1 wk (± 2 days)**	**2 wk (± 2 days)**	**5 wk (± 2 days)**	**6 wk (± 2 days)**
Enrollment and Randomization[1]	X							
Screening[1]	X	X						
Questionnaires[1]		X						X
Blood Draw[2]		X		X		X		X
MRI[3]		X				X		X
Functional Mobiliy[4]		X				X		X
Strength & Central Activation[4]		X						X
3-day food log		X	X	in hosp.	X	X	X	
Accelerometer[A]		X	< -accelerometer on at all times->				X	
Daily Supplement Ingestion**			all start	—>	—>	—>		all stop
Daily Heavy Water Ingestion			all start	—>	half stop	half stop		
*Bilateral Biopsy (muscle & fat)				all subjects	half of subjects[4]	half of subjects[4]		

[1] Performed at the Slocum Center for Orthopedics and Sports Medicine.
[2] Performed at PeaceHealth Labs at Sacred Heart Medical Center, RiverBend. Pre-operative bloods collected per standard of care. If pre-operative blood is collected prior to enrollment, study-specific bloods are drawn before initiating study medication (before – 1wk). Inpatient draws on day of surgery and post-operative day two.
[3] Performed at the the Lewis Center for Neuroimaging, University of Oregon. In case of scheduling, mechanical issues, or patient preference, MRIs may be performed at the Slocum Center for Orthopedics & Sports Medicine. Patients must have all study MRI scans on the same machine.
[4] Performed at the the Center for Medical Education and Research, University of Oregon or the Slocum Center.
[A] Accelerometer ON daily during waking hours (off while sleeping and showers) including during inpatient hospitalization. Between -6wks to -2 wks wear for 7 consecutive days; -1 week before surgery through 2 weeks post-op, wear for 21 consecutive days; 6 weeks post-op, wear for 7 consecutive days.
[B] All pre-operative functional assessments, labs, MRI, strength & central activation, baseline accelerometer and food log must be complete before the subject begins supplement ingestion.
* All subjects will have a single biopsy from each leg in the OR during TKA, while half of subjects will have a single biopsy from each leg at 1 week post and the other half will have a single biopsy from each leg at 2 weeks post. All subjects will only have two biopsies per leg during the entire study. Muscle weight dry versus wet will be compared at 1 or 2 weeks post versus baseline (intraoperative).
**Inpatient Research Protocol & Integration with Standard Patient Care: Protocol while subject is inpatient (IP) on orthopedic floor (estimate 2 to 3 days as IP); Daily AM fasting blood draw by phlebotomist on POD 0 and POD 2. (leave blood on ice, research staff to pick up); Subject to don accelerometer at all times; Twice daily ingestion of supplement (subject and investigators are blinded to treatment group); Ingestion will occur 1 hour after daily physical therapy; Inpatient physical therapy to begin at 9 AM and 1 PM. Therapy per MD/PT standard Tx; Twice daily ingestion of deuterium oxide (D_2O); Daily food-log recordings (all nutrient intake) by subject. Research staff and nursing to remind subject to complete this task.

Specific Aim #2 (Physiology).

Aim #2 - Determine if short-term prevention of atrophy, weakness, and functional mobility leads to positive changes in muscle cell structure and function, and quality of life in the long term (6 months post-TKA) vs. Placebo.

Randomization Assignments.

- Aim #2, Trial #1 enrolled 19 patients before closing enrollment in June 2018. Please refer to the Event Form submitted on June 4, 2018 for additional details. Please refer to study protocol December 2017 for information on Aim #2, Trial #1 EAA proportions and protocols.

- Aim #2, Trial #2 (reflected throughout the protocol as "Aim 2") will consist of 2 groups (N=40/group through protocol completion; EAA vs. Placebo) that are identical in protocol design. Up to 160 patients will be consented to achieve 40 per group completion.

Summary of Study-Related Activities.

- Patient questionnaires before and after surgery
- Functional mobility tests before and after surgery
- Accelerometer before surgery and after surgery
- Optional muscle biopsy before and after surgery

Changes to Study-Related Activities.

Omitted Procedures or Measures for Participants Enrolled in Aim #2

- No strength & central activation testing
- No consumption of heavy water (D_20)
- No muscle biopsies at surgery, 1 week or 2 weeks post-TKA operation
- No blood draws
- No MRIs performed
- No Three day food diary
- Removal of 6 minute walk
- Removal of study questionnaires at 2 weeks post-TKA (functional testing remains)

New or Altered Study Procedures or Measures for Participants Enrolled in Aim #2.

- New study assessment time points: 3 months & 6 months
- Optional (patient choice at enrollment) muscle biopsies at pre-op, 6weeks & 6 months post-TKA
- Option for patients with surgery dates less than 4 weeks prior to surgery to be enrolled in the functional testing arm only
- Option for patients who are taking Coumadin/Warfarin to be enrolled in the non-biopsy arm of the study
- Biopsies will be closed with suture and/or steri-strips
- Addition of the pain and catastrophizing questionnaire (covariate measure)

The procedure schedule can be referenced in Figure 2 (page 23, following a description of all study-related procedures and measures for Aim #2).

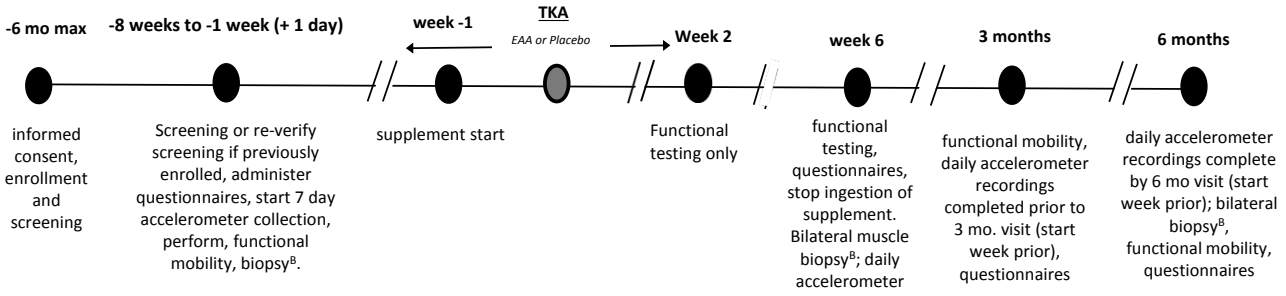
Primary Physiological Outcome Measures. Consistent with the Secondary Outcome Measures described in Aim #1 (please refer to Aim #1) aside from the omitted and new/altered procedures noted above (see Changes to Study-Related Activities in this section), but followed over a longer period time (6 months).

Secondary Physiological Outcome Measures. Consistent with the *Primary Outcome Measures* described in Aim #1 (please refer to Aim #1) aside from the omitted and new/altered procedures noted above (see Changes to Study-Related Activities in this section).

Covariate Measures. Consistent with the *Covariate Measures* described in Aim #1 (please refer to Aim #1 above), with the addition of the pain and catastrophizing scale (questionnaire)

Aim #2 Study Activities Timeline. Participants will be enrolled for up to six months prior to surgery through 6 months post-operatively. Figure 2 (next page) describes the schedule of study-related events, including evaluable time windows, under Study Aim #2.

Overview of Aim #2 study timeline, activities, measures and procedures.



Schedule of Events								
Time	**-6 mo max**	**-8 wks to -1 wks (+ 1 day)**	**-1 wk (± 0 day)**	**TKA (Day 0)**	**2 wk (± 1 week)**	**6 wk (± 1 week)**	**3 mo (± 2 weeks)**	**6 mo (± 2 weeks)**
Enrollment	X	X						
Screening	X	X						
Questionnaires		X				X	X	X
Functional Mobility Test		X			X	X	X	X
Accelerometer		XA				X	XA	XA
Supplement*			start →			Stop		
Bilateral Muscle BiopsyB		XB				X		X

Functional testing & biopsy: Performed at the Dreyer Lab, University of Oregon or the Slocum Center.

A Subjects will have the accelerometer ON daily during waking hours (off while sleeping and showers) for a 7 consecutive day period ending at this time point.
BOptional muscle biopsy as per patient choice; Biopsies must be completed a minimum of 4 weeks prior to TKA; patients who are enrolled between -4 weeks and -1 week (+ 1 day) will not be eligible to participate in the biopsy arm.
*Twice daily ingestion of supplement (subject and investigators are blinded to treatment group); Ingestion will occur within1 hour after daily physical therapy.

Participant Withdrawal.

Participants will be withdrawn from the study by the PI or physician Co-Investigators if required to protect the subject's well-being. Adverse events will be closely monitored and assessed for possible relationships to study treatment. Please refer to the *Adverse Event* form, located in Appendix 2. Clear study-stop criteria have been established:

Study Stopping Criteria

Criterion #1: Patient experiences a serious reportable adverse event (SRAE).

1. Death; immediate individual stop.
2. Life-Threatening; immediate individual level stop.
3. Hospitalization (initial or prolonged)
    a. Where prolonged is defined as discharge on Post-Operative Day 4 (POD #4) or longer; use Criteria #2 to further assess for study stop.
    b. Where immediate subject withdrawal from study will occur at Post-Operative Day 6 (POD #6)
4. Disability or permanent damage; immediate individual stop.
5. Required intervention to prevent permanent impairment; Use Criteria #2 to further assess.

Criterion #2: The intensity of an adverse event warrants discontinuation at the individual level.

For all adverse events (not limited to SRAEs), grading of severity is derived from the daily life consequences and need for additional treatments/interventions.

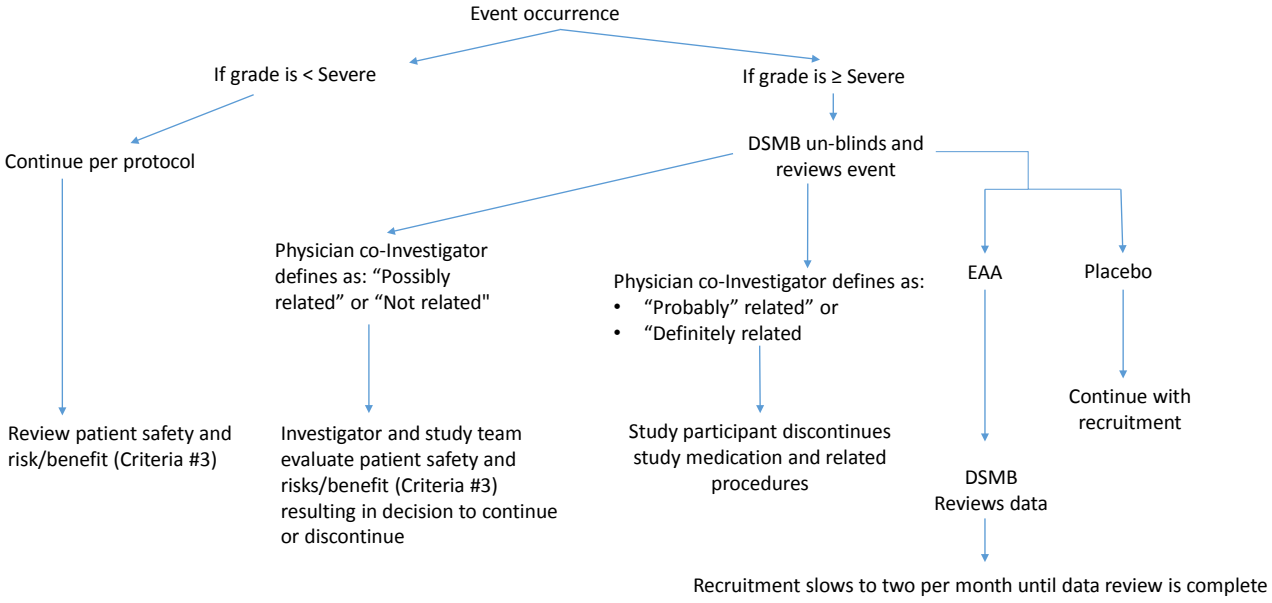
Intensity of adverse events are defined as follows:

- Mild – Does not interfere with daily activity
- Moderate – Interferes with daily activity, no treatment required
- Severe – Prevents daily activity or requires treatment (see decision support below)
- Life Threatening – Supports stopping decision

Please refer to Figure 3 (next page).

Figure 3. Algorithm for individual-level study discontinuation, essential amino acid versus placebo in TKA.

Algorithm for individual-level study discontinuation.



Criterion #3 – Risk/benefit criteria for individual-level study stop.
Subjects with adverse events will be assessed for risk/benefit stopping criteria.

- Subject experiences clinically relevant deterioration in health (additional adverse events, vital signs, laboratory parameters) following the initial adverse event
- Subject demonstrates clinically relevant change in liver or renal parameters, as confirmed by a minimum of one additional repeat comprehensive metabolic panel, occurring no less than 12 and no greater than 24 hours following initial report.
- Subject demonstrates clinically relevant change in vital signs if technical failure or human error can be excluded, supported by at least two clinically relevant measures.

Criterion #4 – Cohort-level study stop.
All serious adverse events will be reported to the DSMB and IRBs within 24 hours of any member of the study team becoming aware of the adverse event.

The following will be assessed in consideration of complete trial discontinuation:

1. The types of adverse events
2. Individual-level adverse event frequency and intensity
3. Ability to effectively monitor observed adverse events in current and future patients
4. Reversibility of adverse events and possible outcomes
5. The number of subjects who experience (a) the same adverse event and (b) any adverse events
6. Medication assignment among subjects who experience (a) the same adverse event and (b) any adverse events

Stopping Rules Adapted from: Sibille M, Patat A, Caplian H & Donazzolo Y. A safety grading scale to support dose escalation and define stopping rules for healthy subjects first entry into man studies. British J Clin Pharm 2010; 70(5): 736-748.

Data Collection.

Patient data for all study-related activities will be maintained on paper source documents stored in the Slocum Foundation office at the Slocum Center for Orthopedics & Sports Medicine. Each patient will have a dedicated patient binder for tracking all study-related activities. Data will be abstracted from the paper source documents and/or electronic health records (Slocum Center and PeaceHealth) and entered onto an electronic spread sheet (e-Case Report Form, CRF). Data abstracted onto the eCRF will include a patient identifier (no names) and all data points captured during the patient's enrollment.

The following source documents will be updated and reviewed with the patient at each study time point:

- Health history, new health events (updated by study coordinators)
- Medication log (updated by study coordinators)
- Study medication log (completed by patient)
- D_20 intake log (completed by patient, Aim #1 only)

The study logs can be reviewed in Appendix 2.

Sources of Materials.

Patient history, body composition, strength and functional data, blood samples, and muscle & fat tissue samples will be obtained specifically for research purposes. Coded data will be stored on computers that are password protected and in locked offices. Data on paper will be stored in files in a locked file cabinet (printouts) and computers in a key-code-access-only lab in a badge-access building. All data and material collected will be coded. Codes linked to subjects' names will be stored in the PI's office (key lock) on a MacPro with password protection and backed up to a protected and locked time capsule 2T external storage device. All MR images will be transferred as DICOM files to Dr. Dreyer's laboratory via encrypted electronic data transfer protocol currently established by the collective efforts of our IT department at the University of Oregon.

All research personnel will have access to the coded data, and coded blood and coded tissue specimens. Only the PI, physicians, and study coordinators will have access to the identifiable private information. Only the study coordinator will have access to patient coding.

Please see ***Data Elements Table*** (Appendix 1) for a list of all elements needed to complete the aims of this trial.

Data Analysis.

Keith Smolkowski, PhD, will be responsible for the statistical analysis of this project. He will be responsible for the statistical modeling and, along with Lisa Strycker, MA, will analyze all data points in our study (Oregon Research Institute).

- **Aim #1:** Aim 1 will compare differences between the two groups (EAA vs. Placebo) across experimental conditions in the change in post-TKA metabolism (synthesis & breakdown) from baseline to 1 and 2 wk post-TKA.

- **Aim #2 (trials 1 & 2):** Aim 2 will also compare measures of functional mobility with muscle cell structure (CSA) and mitochondrial function (respiration), including accelerometer and quality of life measures.

Analysis of covariance. ANCOVA will be used to answer research questions for aim 1 & 2. ANCOVA maximizes statistical power by incorporating the pretest measure as a regression-based control, reducing individual variance. Power improves as the pre-post correlations increases. Our preliminary data indicate that pre-post correlations for many key endpoints are highly correlated (e.g., muscle volume r = .96 for operative leg and r = .97 for non-operative leg; strength r = .42 to .89; stair-climb down time r = .78).

Power analyses. For our power calculations, we have assumed a Type I error rate (α) of .05, two-tailed. We based power calculations on effect sizes (d) that we derived from analysis of preliminary data preliminary data and prior studies (10, 30, 32, 33, 36, 41, 51, 113). We calculated d as the standardized mean difference between treatment conditions at follow-up: $d = (x_C - x_T)/S$, where x re presents the primary outcome mean in the Placebo and EAA conditions and S is the pooled standard deviation, after adjusting x for pretest scores and covariates (sex, tourniquet time, age, body mass index, fat/carbohydrate/protein intake).

We propose to enroll **40 total subjects for Aim 1** who are retained through protocol completion (20/group, EAA and Placebo, randomized to either 1 week or 2 week post-operative biopsies), and **80 total subjects** (40/group, EAA and Placebo) **for Aim 2** who are retained through protocol completion to account for possible attrition and to ensure sufficient power to test the hypotheses that EAA therapy up-regulates basal levels of muscle protein synthesis, and reduces post-operative atrophy bilaterally, preserves quadriceps strength, and improves functional mobility relative to a Placebo condition.

Effect sizes. Effect sizes were as follows: (a) Cell and FSR data: REDD1 d = 1.24, SLC36A2 d = 1.27, and FSR d = 1.51 (FSR calculated from mean change from baseline). The mean effect across outcomes was d = 1.37, reflecting large effects. (b) Physiological and functional data (percent change from Baseline to 6 wk): operative quadriceps volume d = 1.52, and isometric strength d = .90; non-operative quadriceps volume d = 1.05, and isometric strength d = .66; timed up-and-go test d = 1.03, timed stair ascent d = 1.08, timed stair descent d= 1.13, and six-minute walk test d= .70. Mean effect size was d = 1.01, reflecting large effects.

Power calculations. Power calculations were based on the sample size and minimally detectable ES (MDES) as follows:

- **Aim #1:** For a sample size of 20 completed subjects/group (10/group for metabolic studies, however, EAA vs. Placebo = 20 total EAA and 20 total Placebo).Aim1, proposed study can detect

ESs of d > 1.10 with 85% power, assuming no pretest covariate, and the minimally detectable ES drops to 0.95 or 0.72 with pretest covariates of r = .50 or .75, respectively. The preliminary results show ESs averaged 1.37 for Cell and FSR outcomes, and all exceeded .95. The proposed study would thus have the power to detect anticipated condition effects for Cell and FSR outcomes in Aim 1 with 10 subjects per condition. We can also detect moderation effects for sex if the associated interaction accounts for at least 21% to 31% of the variance, assuming covariate of r = .75 or .50, respectively.

- **Aim #2:** For a sample size of 40 completed subjects/group (EAA and Placebo), the proposed study can detect ESs of d > .79 with 85% power, and the minimally detectable ES drops to .68 or .52 with pretest covariates of r = .50 or .75, respectively. Preliminary results show an average ES of d = 1.01 across all Aim 2 outcomes, so this study will have power to detect anticipated physiological and functional effects with 40 subjects per condition. We can detect condition-by-sex moderation effects if the associated interaction accounts for at least 12% to 19% of the variance, assuming covariate of r = .75 or .50, respectively. The tests of sex-by-condition interactions in both aims are likely underpowered for some important effects, so we will also consider the size of the effect, even if not statistically significant, as an indicator of potentially important sex-related differences.

Random coefficients analysis. Keith Smolkowski, PhD, will run random coefficients analysis (RCA) to model muscle, strength, and functional mobility across time as well as data points generated from accelerometer and functional analysis. RCA models trajectories from assessments nested within subjects (48); tests of condition are represented by the interaction between a time factor and treatment condition. The RCA avoids the many pitfalls associated with traditional repeated measures ANOVA (52, 91, 101). RCA adjusts for within-individual dependence or autocorrelation in the data (48, 91, 108), and with four measurements, the RCA can model nonlinear growth. It does not require fixed spacing among assessments, accommodates missing values over time, and allows piecewise models (i.e., splines) (48). RCA provides a more powerful analysis than ANCOVA. Power estimates of growth models, however, require several assumptions that are not easily established. Thus we rely on the fact that the power for RCA has been shown to surpass that of ANCOVA (53, 86) especially when applied to four or more assessments (118). The RCA, then, will detect smaller differences between conditions than the mixed-model ANCOVA. Finally, optimal Type I error rates do not depend heavily on normally distributed data (48, 52, 74, 85). These methods are also appropriate for relatively small samples, such as those planned for the present study (74).

Missing data. Most longitudinal trials result in missing data. The proposed methods will reduce bias due to missing data (6, 73). Maximum likelihood methods that model time as random, such as RCA, use any available data across time, reducing bias and increasing power (43, 91). Our preliminary data show that we have been successful in collecting >92% of all data points. However, to minimize missing data, the study coordinator and staff will devote significant time and effort to data collection and retention of study subjects.

Potential Limitations.

We do not expect problems with the proposed experimental design or data collection. Our research team has performed every test and used each method included in this proposal. We have identified and addressed issues that may present potential problems and proposed solutions in the Potential Problems and Solutions section of Aim 1. We are well aware of the potential issues related to enrollment goals

and attrition when conducting a clinical trial. Our subjects are older adults who are undergoing a major operation. However, our preliminary studies are evidence that we are very experienced with this patient population. The average number of TKA patients who complete our studies is 3-5/mo. We are therefore very confident that we will be able to complete studies on 40 TKA patients (Aim 1) and 80 TKA patients (Aim 2); 120 over the entire 5 year study. Issues that present potentially unique problems to our longer-term study are addressed below.

Statins**.** A potential limitation with long-term muscle cell function of our clinical study is concurrent use of statins, which have a strong potential to compromise muscle mitochondrial function (7, 68, 109, 117). Over 50% of our TKA subjects use statins. Mitochondrial dysfunction may introduce sample bias as well as negatively impact short and longer-term function. To account for this, we propose to measure mitochondrial respiration using the Oroboros O2k respirometer, and normalize results statistically and use the n0n-operative leg as internal control. Thus, measuring mitochondrial respiration will allow us to determine the effects of muscle preservation and accelerated recovery of functional mobility (EAA) while accounting for statin use between groups as well as between sex and over time statistically.

Research Population & Recruitment Methods

Study populations for Aim #1 and Aim #2 are identical. In addition, the recruitment process does not differ between the short-term, biochemistry study (Aim #1) and the longer term, physiologic study (Aim #2).

Target Population.

Both aims will target older adults, ages 50 – 80 years (Aim 1) and 40 – 80 years (Aim 2) for have elected to undergo primary total knee arthroplasty performed at PeaceHealth Sacred Heart Medical Center at RiverBend or Slocum Ambulatory Surgery Center by Dr. Jewett, Lantz, Mohler or Shah. Between 2005-2010, they performed a total 2,066 TKAs. Of those, 1,406 of the patients were between 50-80 years of age (68%). A total of 120 subjects will be enrolled in the two study aims.

- **Aim #1.** The biochemistry study aim will obtain complete data on **40 subjects**, randomized to either EAA supplementation or placebo, and further randomized to receive 1 week or 2 week post-surgical biopsies. Randomization is stratified by gender. Up to 70 subjects will be enrolled to account for withdrawal and attrition.
- **Aim #2.** The physiology study will obtain complete data on **80 additional subjects**, randomized to either EAA supplementation or placebo. Up to 160 subjects will be enrolled to account for withdrawal and attrition.

The target population will not include pregnant women as the study population are patients who have scheduled a total knee arthroplasty. Patients will not be scheduled for surgery if they are pregnant as per standard of care. The point in which study coordinators approach patient for recruitment, they have already been scheduled for TKA. Furthermore, women of potential childbearing age will be asked if they are or could possibly be pregnant. A hormone pregnancy test will be administered to those women who cannot verify pregnancy status prior to ingestion of amino acid supplement. Women who are surgically sterile (e.g., hysterectomy); or who are otherwise incapable of pregnancy (e.g. menopause) will not have to undergo pregnancy testing.

Inclusion & Exclusion Criteria.

Study Aim #1

Inclusion Criteria.

- Patient is 50 – 80 years old
- Patient is having a primary, unilateral TKA
- Patient has signed the current, IRB approved informed consent document

Exclusion Criteria.

- Patient has previous TKA and/or total hip arthroplasty surgery on ipsilateral lower extremity.
- Patient has previous TKA on contralateral leg
- In the judgment of the physicians the patient is not anticipated to have contralateral TKA before end of treatment is completed (6 weeks Aim #1)
- Patient has dementia or related mental issues that may potentially put the subject at risk as determined by the surgeon.
- Patient has untreated endocrine disease (hypo/hyper thyroidism, Addison's Disease or Cushing's syndrome, etc.)
- Patient has uncontrolled diabetes; defined by lab result of hemoglobin A1c level > 8.0 mg/dL at the pre-anesthesia testing visit, which must occur before the patient begins the study medication or heavy water ingestion.
- Patient has significant heart, liver, kidney or respiratory disease.
- Patient has peripheral vascular disease.
- Patient has active cancer.
- Patient has taken anabolic steroids in the prior six months.
- Patient has known current alcohol or drug abuse.
- Patient has conditions that prohibit Magnetic Resonance Imaging (MRI).
- Patient discharge status is known to be to care facility.
- Patient is on oral blood thinner such as Warfarin, Coumadin, Eliquis, etc.

Study Aim #2

Inclusion Criteria – Biopsy Participant.

- Patient is 40 – 80 years old
- Patient is having a primary, unilateral TKA
- Patient has signed the current, IRB approved informed consent document
- Patient can schedule pre-operative biopsy at least 4 weeks prior to date of TKA

Exclusion Criteria – Biopsy Participant.

- In the judgment of the physicians the patient is not anticipated to have other lower extremity total joint arthroplasty before end of treatment is completed (6 months).
- Patient has had previous lower extremity total joint arthroplasty surgery within 6 months of starting -8 week to -1 week (+1 day) study activities.
- Patient has dementia or related mental issues that may potentially put the subject at risk as determined by the surgeon.
- Patient has untreated endocrine disease (hypo/hyper thyroidism, Addison's Disease or Cushing's syndrome, etc.)

- Patient has uncontrolled diabetes; defined by lab result of hemoglobin A1c level > 8.0 mg/dL. ~~at~~ If the patient has consented to biopsies, confirmation of the first step (below) must occur before patient can have pre-operative biopsy. Two-step verification process for this will occur; (1) patient self-report, with the addition of any available medical records Slocum Orthopedics has regarding patient diabetic status, and (2) Upon completion of standard of care pre-operative blood draw, we will confirm results of HbA1c. If the patient has not consented to have biopsies, the same verification process will occur before the patient begins taking the supplement.
- Patient has significant heart, liver, kidney or respiratory disease.
- Patient has peripheral vascular disease.
- Patient has active cancer.
- Patient has taken anabolic steroids in the prior six months.
- Patient has known current alcohol or drug abuse.
- Patient discharge status is known to be to care facility.
- Patient is on oral blood thinner such as Warfarin, Coumadin, Eliquis, etc.

Inclusion Criteria – Non-Biopsy Participant

- Patient is 40 – 80 years old
- Patient is having a primary, unilateral TKA
- Patient has signed the current, IRB approved informed consent document

Exclusion Criteria – Non-Biopsy Participant

- In the judgment of the physicians the patient is not anticipated to have other lower extremity total joint arthroplasty before end of treatment is completed (6 months).
- Patient has had previous lower extremity total joint arthroplasty surgery within 6 months of starting -8 to -1 week (+ 1 day) study activities.
- Patient has dementia or related mental issues that may potentially put the subject at risk as determined by the surgeon.
- Patient has untreated endocrine disease (hypo/hyper thyroidism, Addison's Disease or Cushing's syndrome, etc.)
- Patient has uncontrolled diabetes; defined by lab result of hemoglobin A1c level > 8.0 mg/dL. ~~at~~ If the patient has consented to biopsies, confirmation of the first step (below) must occur before patient can have pre-operative biopsy. Two-step verification process for this will occur; (1) patient self-report, with the addition of any available medical records Slocum Orthopedics has regarding patient diabetic status, and (2) Upon completion of standard of care pre-operative blood draw, we will confirm results of HbA1c. If the patient has not consented to have biopsies, the same verification process will occur before the patient begins taking the supplement.
- Patient has significant heart, liver, kidney or respiratory disease.
- Patient has peripheral vascular disease.
- Patient has active cancer.
- Patient has taken anabolic steroids in the prior six months.
- Patient has known current alcohol or drug abuse.
- Patient discharge status is known to be to care facility.

Research Sites.

The study will be conducted at multiple research sites. Patients will be asked to travel to various clinical and research-specific locations to complete study-related activities.

Table 3: Participant Research Sites, Essential Amino Supplementation vs. Placebo in TKA.

Research Site	Location	Study-Related Activities
Slocum Center for Orthopedics & Sports Medicine **Slocum Research & Education Foundation** **Slocum Ambulatory Surgery Center**	55 Coburg Road Eugene, OR	Routine standard of care for TKA patients; participant identification, screening, enrollment, consenting, & follow-up; completion of patient-reported outcome measures; main study-coordination site;; functional mobility tests, strength tests (including central activation) (Aim #1 and #2); alternative MRI site in the event LCNI is unavailable or Slocum is preferred by the patient (Aim #1 only); pre/post-operative bilateral biopsies; outpatient surgery center (Aim #2 only).
Dreyer Laboratory, University of Oregon	Pacific Hall	Analysis of blood specimens (Aim #1 only); function and strength testing; strength & central activation deficit; pre/post-operative bilateral biopsies (Aim #1 and #2).
Lewis Center for Neuroimaging, University of Oregon	Lewis Integrative Sciences Building 1440 Franklin Boulevard, Eugene, OR 97403	MRI (Aim #1 only)
PeaceHealth Laboratories	Various (patient choice possible, RiverBend for pre-admit labs as standard of care)	Blood draws (Aim #1 only).
PeaceHealth Sacred Heart Medical Center at RiverBend	3333 RiverBend Drive Springfield, OR 97477	Routine standard of care for TKA patients plus study-specific procedures: blood draws, food diary population, supplement ingestion, standardized physical therapy procedures (Aim #1 and #2).
NW Compounders	2734 SW Orchard Hill Lane Lake Oswego, OR 97035	Aim 1 only -EAA supplement is being compounded at this pharmacy and shipped to the Dryer Lab. The pharmacy is required per standard of care by

		the Board of Pharmacy to have on file known allergies of the patient. This will be provided from the patients' medical history on file with Slocum Center for Orthopedics & Sports Medicine (Aim #1).

Participant Recruitment.

Patients eligible for enrollment with pre-operative biopsies will be recruited a least four to eight weeks, and no more than six months prior to surgery from the clinics of Dr. Jewett, Lantz, Mohler or Shah, all fellowship trained, board certified orthopedic adult knee reconstruction surgeons. Patients eligible for functional testing only (non-biopsy) may be enrolled with enough time to complete functional testing baseline assessments prior to start of supplement (complete functional testing by -1 week + 1 day).

Primary Recruitment Approaches. In-person consenting will assure all subjects receive a consistent informed consent process and have all questions answered prior to enrollment.

1. Physician Co-Investigators will identify potential subjects for enrollment during a routine clinic appointment that results in a decision by the subject to proceed with total knee arthroplasty. The initial informed consent, screening and enrollment may occur up to six months prior to surgery. The physician will request via the Medical Assistants or Surgery Schedulers ("physician extensions") the study coordinator be contacted to provide study information and complete the informed consent process. Physician extensions will not be engaged in any research-related activities, but will identify subjects as potentially eligible or not based on past medical history, which is disclosed to the physician extension per standard of care. Only the research coordinators or physician Co-Investigators perform informed consenting or formally screen candidates for inclusion/exclusion criteria. (Please see Informed Consent Process below for information about the activities involved in subject consenting).

2. Pre-screen/identify potential subjects for recruitment based on inclusion/exclusion criteria from existing surgical appointment list. Potential subjects would be contacted by phone and asked to make an appointment with the study coordinator to have a thorough discussion of study-related procedures and to obtain written informed consent. Additionally, potential subjects could be mailed a recruitment letter with ability to contact research coordinators to identify themselves as interested in participating. Strong preference will be given to an in-person consenting process; however, should the patient live out of town, a phone consenting process with mailed in executed consent form is allowable. Should a participant elect to mail in the informed consent document, the research coordinator will re-verify all questions have been answered and the patient understands all study-related procedures at the pre-operative study appointment (1 to 6 weeks prior to surgery). No study related procedures will be initiated prior to confirming patient eligibility, understanding and willingness to participate. Patients will have to have an in-person discussion (see Informed Consent Process below) before enrolling into the study.

Secondary Recruitment Approaches.

1. Medical Assistants of participating physician co-Investigators will have access to a study information flier (Appendix 4) which may be distributed to interested subjects, or subjects that in the judgment of the physician Co-Investigator, are potential candidates for enrollment.

2. Information about the study will be included in Slocum Foundation or Slocum clinic newsletters, which will be available in clinic waiting areas. The following text will be used on printed materials accessible to patients for Aim #1:

   Interested in helping Slocum and University of Oregon researchers discover better ways to recover after knee surgery? Consider participating in our Amino Acid Supplementation & Total Knee Replacement Research Study funded by the National Institute on Aging, which is one of the National Institutes of Health.

   The goal of the research is to determine if providing amino acid supplementation (building blocks for protein) can stimulate recovery and reduce muscle loss after surgery. Our preliminary studies suggest taking essential amino acid supplements prior to surgery and for two weeks after surgery helps prevent muscle atrophy.

   To find out more information, please see our website at www.slocumfoundation.org, or ask your physician. At this time, Dr. Jewett, Dr. Lantz, Dr. Mohler, and Dr. Shah are the surgeons participating in the Essential Amino Acids study. You can also come to a study information session at Slocum on the 2nd Wednesday of each month at 12:00p. Please RSVP by calling 541-868-3232 if you plan to attend an information session. A light lunch will be provided.

3. Information about the study will be included on the Slocum Foundation website. The following text will be used on the website:

   Interested in helping Slocum, Oregon Research Institute and University of Oregon researchers discover better ways to recover after knee surgery? Consider participating in our Amino Acid Supplementation & Total Knee Replacement Research Study funded by the National Institute on Aging, which is one of the National Institutes of Health.

   The goal of the research is to determine if providing amino acid supplementation (building blocks for protein) can stimulate recovery and reduce muscle loss after surgery.

   Who Can Participate?
   Patients having total knee replacement surgery performed by Dr. Jewett, Dr. Lantz, Dr. Mohler, or Dr. Shah may be able to participate. Your physician and the study coordinator from the Slocum Foundation will determine whether or not you qualify for the study.

   What's Involved?
   Each participate will be randomly assigned to either receive the essential amino acid supplement or a placebo, which is a small drink without any essential amino acid supplement. Like a coin flip, you have about a 50-50 chance of receiving the real essential amino acid supplement. You would need to take the supplement two times per day, after breakfast and lunch, one week before surgery and six weeks after surgery.

MRI so we can measure how much muscle you have in your legs before surgery.
In addition to taking the supplement, we need to test how strong you are before surgery and how well you can do certain activities. For example, we would ask you to walk as far as you are able for six minutes. You would also be asked to wear a pedometer that measures you level of activity each day and record your food intake in a daily food diary.

While you are in the hospital and under anesthesia, your surgeon will be taking a biopsy of your muscles. After your surgery, staff from RiverBend hospital will collect blood samples each morning before you have breakfast.

You will be asked to have biopsies during and after surgery and repeat many of the study procedures you had before surgery (example: MRI, tests that measure your ability to move around).

A study coordinator from the Slocum Foundation will make sure you understand all the steps and procedures, as well as the risks and potential benefits, involved in the study before you agree to participate.

Has anyone participated in a study like this before?
Yes, Drs. Jewett, Lantz and Shah participated in the pilot data project. The pilot data was published in the Fall of 2013. You can read the study results in the Journal of Clinical Investigation: http://www.jci.org/articles/view/70160.

What can you tell me about the potential benefits of the study?
Our preliminary studies suggest taking essential amino acid supplements prior to surgery and for two weeks after surgery prevents muscle atrophy. Preventing muscle atrophy is important because improving muscle strength and functional after knee replacement may decrease recovery time; however, this is part of what we are investigating. There is a chance you would receive the placebo (no real supplement), in which case you may not benefit from study participation directly, but you would play an important part in helping us determine whether or not EAA supplementation is beneficial.

Who can I contact to find out more information? *You may contact your physician or the Slocum Foundation at 541.868.3232 or email info@slocumfoundation.org.*

4. Monthly information sessions. One time per month the Slocum Foundation will host an information session for potential subjects.

5. Information flier posted in patient exam rooms (Appendix 4).

For Aim #2, the following material will be available on the Foundation website:

Interested in helping Slocum, Oregon Research Institute and University of Oregon researchers discover better ways to recover after knee surgery? Consider participating in our Amino Acid Supplementation & Total Knee Replacement Research Study funded by the National Institute on Aging, which is one of the National Institutes of Health.

The goal of the research is to determine if providing amino acid supplementation (building blocks for protein) can stimulate recovery and reduce muscle loss after surgery.

Who Can Participate?
Patients having total knee replacement surgery performed by Dr. Jewett, Dr. Lantz, Dr. Mohler, or Dr. Shah may be able to participate. Your physician and the study coordinator from the Slocum Foundation will determine whether or not you qualify for the study.

What's Involved?
Each participate will be randomly assigned to either receive the essential amino acid supplement or a placebo, which is a small drink without any essential amino acid supplement. Like a coin flip, you have about a 50-50 chance of receiving the real essential amino acid supplement. You would need to take the supplement two times per day, after breakfast and lunch, one week before surgery and six weeks after surgery.

In addition to taking the supplement, we need to test your functional mobility before surgery and after to see how well you can do certain activities. For example, we would ask you to walk as far as you are able for six minutes. You would also be asked to wear a pedometer that measures you level of activity before and after your surgery.

You will be asked to have one muscle biopsy on both legs before your surgery (four to eight weeks), and again six weeks and 6 months after surgery. You may choose to participate in the study without having the biopsies. In addition, if your surgery date is less than 4 weeks away, you may be eligible to participate in the study and complete functional testing and non-biopsy activities only.

A study coordinator from the Slocum Foundation will make sure you understand all the steps and procedures, as well as the risks and potential benefits, involved in the study before you agree to participate.

Has anyone participated in a study like this before?
Yes, Drs. Jewett, Lantz and Shah participated in the pilot data project. The pilot data was published in the Fall of 2013. You can read the study results in the Journal of Clinical Investigation: http://www.jci.org/articles/view/70160. In addition, the first part of this new study was completed in 2016. In the new study, 40 patients took the supplement and participated in similar study activities for six weeks after surgery.

What can you tell me about the potential benefits of the study?
Our preliminary studies suggest taking essential amino acid supplements prior to surgery and for two weeks after surgery prevents muscle atrophy. Preventing muscle atrophy is important because improving muscle strength and functional after knee replacement may decrease recovery time; however, this is part of what we are investigating. There is a chance you would receive the placebo (no real supplement), in which case you may not benefit from study participation directly, but you would play an important part in helping us determine whether or not EAA supplementation is beneficial.

Who can I contact to find out more information? *You may contact your physician or the Slocum Foundation at 541.868.3232 or email info@slocumfoundation.org.*

Informed Consent Process.

Research participants will be presented with a thorough description of all study-related procedures in-person. Participants will be required to read, review, and discuss the study with study coordinators prior to executing the informed consent document. Study coordinator review of the ICF will include reviewing all of the bullets on the last page of the consent form with the patients and ensuring participants understand the document fully before authorizing consent.

Participants will have an opportunity to ask their surgeon or the study coordinators any questions prior to obtaining informed consent. No study-related procedures will be initiated prior to obtaining written consent. To accommodate out of town patients, this protocol allows for consenting conversations to happen by phone, and patients who agree to participate may mail in their executed ICF. However, patients who elect this option will be required to review study procedures and verbally re-consent on the day of their screening, before any study related procedures are initiated.

Surgeons will have responsibility for approving participants for study entry, specifically for ensuring study inclusion and exclusion criteria have been met. If a study subject has questions related to study procedures at the Dreyer Lab, Lewis Neuroimaging Center, the study coordinator will answer participant questions. In the event the study coordinator does not know the response or for which more expertise is necessary to be fully addressed, the study PI will be contacted, and all questions answered in advance obtaining participant consent.

In addition to the Informed Consent Form, participants will also be asked to sign a HIPAA Authorization form which allows information to be transferred from HIPAA-compliant entities (e.g. hospital and clinic) to the research team.

Enrollment & Randomization.

The study coordinators will communicate participant enrollment to the study PI through a secure, encrypted electronic study management interface hosted by the Slocum Foundation, under the information technology physical and technological safeguards of the Slocum Center for Orthopedics & Sports Medicine. The PI will retain a list of Patient Identification Numbers (PID), which will randomly assign subjects to EAA or placebo, ensuring blinding of participants, research staff and study statisticians. The PI will disseminate supplement to the research coordinators to ensure blinding.

Following enrollment, before supplement ingestion begins, patients will be provided with a Study Patient Binder. The Study Patient Binder will provide patients with instructions regarding supplement and/or heavy water administration, accelerometer use, and food diary entries. The Study Patient Binder will also provide patients with directions to testing locations (e.g. labs, MRIs).

Participant Compensation.

Participants are asked to spend extra time and travel to various research sites to complete study-related activities. Participants who complete the study will receive:

- **Aim #1:** $300.00 payable upon completion of week 6 study activities (study completion), but prorated at the following schedule:

- $50 for completion of one or more -6wk study events
- $50 for completion of one or more -1wk study events
- $50 for completion of one or more day of surgery study events
- $50 for completion of one or more 1 week post-op study events
- $50 for completion of one or more 2 week post-op study events
- $50 for completion of one or more 6 week post-op study events

- **Aim #2:** Maximum of $300.00 payable upon completion of six-month post-TKA study activities (study completion), but prorated at the following schedule:

Compensation plan with biopsies:

- $100.00 for completion of preoperative biopsy
- $100.00 for completion of six week postop biopsy
- $100.00 for completion of six month postop biopsy (study completion)
- No compensation for two week and three month study activities

- **Compensation plan without biopsies:**
  - $20.00 for completion of preoperative testing
  - $20.00 for completion of two week postop testing
  - $20.00 for completion of six week postop testing
  - $20.00 for completion of three month postop testing
  - $20.00 for completion of six month postop testing (study completion)

Should a participant not reach one of the defined compensation endpoints above, the amount of remuneration will revert back to the last completed interval (no pro-rating between study intervals). For example, a participant in Aim #1 who completes one or more study activities on day of surgery would receive $150.00. For Aim #2, if a patient completes six week postop biopsy would receive $200.00

Provisions for Participant and Data Confidentiality.

Study Team Training.

All key personnel have completed and passed the certification exam of the NIH-mandated course on protection of human subjects. The screening tests will allow us to exclude a priori subjects with potentially higher risk of developing complications. All methods and treatments within this proposal are IRB approved and have been tested for feasibility, patient tolerance, and adverse reactions. Identifiable issues have previously been eliminated. Protections against risks for all subjects in the proposed study will be as follows:

Technological and Physical Safeguards.

To minimize this risk, only the investigators, study personnel, research sites, and the Medical Records department of the Sacred Heart Medical Center at River Bend and Slocum Center will have access to all data containing personal information. As a participating research site, Northwest Compounders will have access to patient names and allergies, which are required to dispense the study medication by law (Aim 1). As a HIPAA covered entity, Northwest Compounders is obligated by law to follow the technological and physical safeguards consistent with healthcare standards for privacy and confidentiality (Aim 1). No coded or de-identified data and/or samples will be provided to anyone outside of the research team.

Slocum Center clinical staff, who routinely access PHI as part of routine business operations will provide the research team with de-identified files, containing a unique research-specific identifier for tests/procedures performed at Slocum . Only study staff directly involved in the administration of patient care or study-specific procedures or testing will have access to identifiable health information.

Confidentiality risks are minimized through our approach and data security practices. All de-identified, coded data will be stored on a secure, encrypted server, housed within the Slocum Center for Orthopedics & Sports Medicine. Slocum Center administers the highest level of technological safeguards the industry offers. The study-specific server is entirely dedicated to the Slocum Research & Education Foundation, sharing no networks with the Slocum Center. The software used to manage study patients allows for multiple levels of data sharing and permissions. For example, study coordinators and team members involved in direct coordination of patient care will have access to identifiable patient information, while collaborating statisticians will have access to only de-identified data files. In addition, any provider information contained in the eCRFs will be limited to a physician ID number only.

Patient binders will be stored behind a limited access, key-entry door, further protected by the biometric finger sensors required to access Slocum after-hours. Because the primary study coordination team (Owen and Kirkpatrick) are housed within a HIPAA compliant, highly secure data protection environment, research data are protected by the safe physical, technical and policy protections as patient medical records.

Finally, a single laptop will be used to capture accelerometer data in the field (e.g. hospital, Dreyer lab). The laptop has multiple fail-safes, which secure the data even if the laptop should be stolen. Using a total hard-drive encryption software program, the laptop will not power on without first entering the encryption key. Accelerometer data are further protected by using only the unique patient identification number when downloading data offsite.

Upon study completion, licensing of the PAM tool requires transmission of de-identified, limited data sets to Insignia Healthcare. Data will be transferred through a secure, encrypted FTP upload. Transferred files will contain only summary level information (e.g. consistent with a limited data set and containing no full sets of patient reported outcome questionnaires). Only the rolled up result data will be transferred to Insignia to fulfill licensing requirements.

As is standard with a clinical trial, information about participation in the research study, including the individual having consented to study participation will be included in the Slocum medical record. When the study coordinators are tracking participation, they may note any important health events and correspondence with the patient in the electronic medical record at Slocum. The patient's surgeon needs this information documented to manage care and monitor their patient's health and well-being.

Potential Research Risks or Discomforts to Patients

Risks.

This is a moderate risk study. Potential risks for all subjects participating in this clinical research project are as described below.

Confidentiality.

Disclosure of personally sensitive data is a risk of clinical investigations involving human subjects. All subjects will sign a HIPAA form acknowledging the PHI included in data collection. Staff at Northwest

Compounders will know research participants by name and document allergy information, as required by law (Aim 1). Northwest Compounders is study medication per standard of care and retains limited patient information as a result (Aim 1).

A copy of the laboratory results collected for study-related procedures will be provided to the patient's primary care provider. Transmission of results will occur under standard HIPAA protocols for clinic to clinic sharing of information (e.g. fax). The patient's primary care provider may elect to follow-up on results to assure high quality care and care management. Patients will authorize lab result sharing through the study-specific HIPAA Authorization for Research.

Under the agreement with MEND Nutrition, Inc., aggregate, de-identified study data will be shared back with MEND, limited to functional testing results and patient reported outcome measures (questionnaires) only. No patients will be directly identifiable through aggregate data, and any cells with resulting counts less than 5 in a table will be omitted to protect patient confidentiality.

Physical injury.

Physical injury (including falls) is a risk when being asked to perform exercise/strength assessment and/or physical independence/performance measures. This risk is increased for persons TKA surgery because of issues related to the surgery and the potential need for assistive devices.

Blood draws (Aim 1 only). The total amount of blood drawn for the purposes of the study will be depend on the study. The potential risks include infection at the site of the blood draw. Careful sterile technique will minimize the chances of infection at the blood draw site. There is a risk for redness, itching, bruising and soreness at the blood draw site. Your blood will be drawn from a site on your arm. Additional blood may be drawn during surgery as determined by the hospital staff and/or doctors outside the purpose of this research study.

> **Aim 1** (N=40, 20/group) and **Aim 2** (N=80, 40/group) will have the same total amount of blood drawn, at study intervals.

Muscle biopsy. Biopsies will be collected from all TKA patients (Aim 2 patients will have the choice to participate with or without muscle biopsies).

- **Aim 1** (N=40, 20/group) will have 1 muscle and 1 fat biopsy per leg during TKA (in the OR) (N=40) and at 1 week post-TKA (N=20); or 2 weeks post-TKA (N=20).
- **Aim 2** (N=80, 40/group) will have 1 muscle biopsy per leg at Baseline, 6wk & 6mopost-TKA.

The biopsies will be obtained from the vastus lateralis using a 5mm Bergstrom biopsy needle with applied suction under sterile conditions. A single incision of 0.5 cm will be made in the skin down to the level of the fascia, and approximately 150 mg of muscle tissue will be removed during each biopsy.

The potential risks are pain, bleeding, infection, and loss of superficial sensation (light touch, hot/cold) to an area around the biopsy site. There will be a scar approximately 0.6 cm long. The risk of bleeding from the biopsy site is 0.2%; the risk of hematoma is 1.4%; the risk of infection is so small that the precise number is unknown; and the risk of a loss of superficial sensation is 0.5% (55). After the biopsy,

there is a 50% chance that the subject will experience pain at the biopsy site for 24- 48 hrs. However, over the counter pain medications have been successful in mitigating pain from the biopsy site.

Aim 1: It is important to note the biopsies are in a fasting state (no food since dinner the night before). For the first biopsies in the hospital on the day of surgery, fasting is required per routine care before surgery. For the second biopsies, occurring at one OR two weeks after surgery, fasting may lead to some discomfort. Patients may have to delay taking their pain medication, or risk upset stomach if they take their pain medication without food.

Aim 2: It is important to note the biopsies are in a fasting state (no food since dinner the night before). Fasting may lead to some discomfort. Patients may have to delay taking their pain medication (if taking any), or risk upset stomach if they take their pain medication without food.

Risk from heavy water (Aim #1 only). Brief periods (< 30 minutes) of dizziness have been reported in a small number of people (< 5%) after drinking tracer water. This is due to the tracer water being "heavier" than normal water. "Heavier" water can temporarily disrupt the inner ear's ability to control balance, producing a sensation of dizziness.

Magnetic stimulation (Aim #1 only). Magnetic stimulation of the peripheral nerve is a safe and non-invasive way of supplying information to the muscle. Because this pulse is magnetic, and not electric, the procedure is painless. No long-term side effects have been reported; however, on rare occasions, individuals find the stimulation uncomfortable.

Magnetic Resonance Imaging (MRI, Aim #1 only). The U.S. Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we will carefully observe those guidelines. For the exposure in our ongoing study, no negative effects have been seen. Some people may feel uncomfortable or anxious during the MRI. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste, or feel tingling sensations or muscle twitches. These sensations usually go away quickly. MRI poses some risks for certain people. If an individual has a pacemaker or some metal objects inside the body, the strong magnets in the MR scanner may do harm. Another risk is a metallic object flying through the air toward the magnet and hitting the subject.

Risk from falls. There is a possibility that a participant may fall and hurt yourself during this study. Knee pain may impact a participant's ability to control muscles. It takes time to heal from this type of surgery and we are asking you to perform physical tasks that may be challenging to you. You will be supervised at all times when completing the tests that help us determine your ability to move and your strength.

Pregnancy risk. The risks of the amino acid supplement to the human embryo or fetus have not been studied. Women who are pregnant are not eligible to participate in this study. Women of potential childbearing age will be asked if they are or could possibly be pregnant. It is possible a hormone pregnancy test will be administered to verify pregnancy status.

Adequacy of Protection Against Risks.

Participant Confidentiality. Described in the Confidentiality section above.

MEND Nutrition, Inc. MEND has the potential as a for-profit supplement company to benefit directly from the outcome of this research. We have purchased the supplement from MEND because MEND's proprietary approach to masking the bittering taste associated with EAAs overcomes a significant barrier to enrollment. MEND is not paying for the study, involved in study design, or execution. MEND has no control over the publication/dissemination of study results. There is no difference between researching EAA supplementation provided by MEND versus another commercial supplement provider such as GNC.

The physicians on this study are not receiving compensation from MEND. Should the nature of this relationship between MEND and any member of the research team (including physicians) change, formal submission per IRB requirements will be executed.

Physical injury. To minimize this risk each subject will be supervised by a member of the research team at all times during testing. All aspects of this proposal are currently used and implemented by the study team.

Blood draws (Aim 1). Each blood tube collected is between 2.7mL and 7.0mL, depending on the blood test requirements. The amount of blood collected at each blood draw is between 28mL and 35mL (high estimate). As reference, when a person donates blood, 500-550mL of blood are donated. Even with seven tubes of blood, the amount collected at each draw is approximately 7% of the amount required at a typical blood donation.

Muscle biopsy. Pain during tissue sampling will be eliminated by anesthesia. Moreover, pain-control measures initiated postoperatively by the research team will be sufficient to control for potential pain at the biopsy sites during inpatient hospitalization (TKA). However, over-the-counter medications such as ibuprofen are sufficient to control such discomfort for all subjects as needed and after the single bilateral biopsies performed at the PI's lab. The risk of bleeding will be reduced with manual direct pressure for 10 minutes or until bleeding has stopped. The risk of infection is minimized by the use of a sterile field. We have performed numerous muscle biopsies in the OR (10, 100) as well as post-operatively at 6 wk post-TKA without incident. Due to the necessity of a fasting state for the one OR two week post-operative biopsies (Aim #1) the study team will accommodate as early as 6:00am for scheduling the biopsies to help minimize any potential discomfort.

Heavy water (Aim #1). Our study coordinators will continually remind subjects via telephone or in person of the potential for balance issues with deuterium oxide (heavy water).

Magnetic stimulation (Aim #1). Participants will be asked to complete a standard Transcranial Magnetic Stimulation Safety Screening Questionnaire. In the event that a subject answers "yes" to one of the questions on the questionnaire, magnetic stimulation will not be used with these participants. If a subject experiences discomfort at any point, this portion of the protocol will be stopped immediately.

There is significant overlap with regards to TMS and MRI safety screening questionnaires (e.g., Do you have a pacemaker?), so, in most cases, people who answer "yes" to a question in the TMS safety screening would be excluded from the study, as they would not qualify for an MRI.

Magnetic Resonance Imaging. All participants will be asked to complete a standard MRI safety screening questionnaire. If it is deemed unsafe for an individual to have an MRI, they will be excluded from the study (see exclusion criteria).

All of the above testing and procedures will be performed either at the hospital where the TKAs are performed (Sacred Heart Medical Center, River Bend), in the PI's lab (which is immediately adjacent to the Sacred Heart Medical Center, University District's Emergency Department), or in the Lewis Center for Neuroimaging (LCNI, location of MRI) (which is 0.5 miles from the Sacred Heart Medical Center, University District's Emergency Department), or Slocum Center for Orthopedics & Sports Medicine located in Eugene.

Risk from falls. Participants will be supervised at all times when completing the tests that help us determine your ability to move and your strength.

Pregnancy risk. Women who are pregnant are not eligible to participate in this study. Women of potential childbearing age will be asked if they are or could possibly be pregnant. It is possible a hormone pregnancy test will be administered to verify pregnancy status.

Data Safety Monitoring Plan

The ultimate responsibility for data and safety monitoring rests with the Principal Investigator. It is equally clear that the institution must promote an environment that facilitates detailed ongoing review and safe conduct of high-priority research studies. Effective data and safety monitoring is not the result of a single committee or individual, but is a shared responsibility within our protocol management scheme. The risk level associated with this study is estimated to be moderate and reasonable.

Plan for Monitoring and Safety Review.

- Hans Dreyer, PhD, PT, the Principal Investigator, will be responsible for monitoring the safety environment of the participants during the entire study. The orthopedic surgeon Co-Investigators Brian Jewett, MD, Brick Lantz, MD, Craig Mohler, MD, and Steven Shah, MD, will be medically responsible for the subjects' care relative to the surgery and post-operative medical coverage.

- Older subjects will be patients of the Sacred Heart Medical Center, continuously monitored by nursing and hospital staff during inpatient stay after TKA, and followed by the surgeons and research team.

- Thorough monitoring of the recruitment, enrollment, retention, informed consent process, adverse events, study procedures, and evaluation of primary and secondary endpoints will be closely observed by the PI, Hans C. Dreyer, PT, PhD., and Co-Investigator Brian A. Jewett, MD.

- DSMB: We have designed a data and safety monitoring committee and plan. The members of the DSMB will be:

- Carmen Castaneda-Sceppa, MD., PhD
  Professor and Director, Graduate Programs in Exercise Science
  Department of Health Sciences

- Subashan Perera, PhD
  Associate Professor of Medicine
  Co-Director & Senior Statistician, Data Management and Analysis Core of the Pittsburgh Claude D. Pepper Older Americans Independence Center

- Kevin Yarasheski, PhD
  Professor of Medicine, Cell Biology & Physiology, and Physical Therapy
  Assistant Director, Biomedical Mass Spectrometry Research Laboratory

Committee members will participate in the completion of the DSMB Charter, Guidelines, assuring all DSMB items identified by NIH via the DSMB Checklist are satisfied.

Adverse Event Reporting. Adverse Event Grading Scale as defined by the AE reporting form and study stop criterion (See Study Forms, Appendix 2). Dr. Dreyer, the Principal Investigator, will be responsible for monitoring and reporting the occurrence of adverse events throughout the study. Subjects will be monitored continuously throughout each testing session, and will be followed by phone or email to verify compliance with study protocol and to monitor for any un-anticipated events.

Anticipated Adverse Events discussed in the Informed Consent Form for this study will include: potential pain, bruising, and/or infection from the blood draws and muscle biopsies and during functional and strength testing. Should an adverse event occur, the Co-Is will be immediately notified and a note will be entered in to the subject's study record using the grading scale listed below.

The research team will follow the IRB Adverse Event Policy on mandatory reporting of Serious Adverse Events (SAEs), and will also report them immediately both orally and in writing to the IRB Program Director or RSA within 24 hours of occurrence or recognition. The PI, Hans Dreyer, PT, PhD, will be responsible for all AE and SAE reporting.

Annual report. Dr. Dreyer will review the protocol on a continuing basis for subject safety and include results of the review in the annual progress reports submitted to the IRB and the appropriate NIH agency as necessary. The annual report will be provided to the Office for the Protection of Human Subjects and will include a list of adverse events.

Content of annual report. The annual report will address: (1) number of adverse events and description of the event; (2) reason for dropouts from the study; (3) number of participants in the study; (4) whether the study should be continued based on the current data collected and the need to achieve the aims stated.

Potential Research Benefits to Participants

The proposed research is based on strong preliminary evidence that EAA reduce post-operative muscle atrophy by 3-fold in the operative and non-operative lower extremities. Further in our pilot studies,

these positive results extended to gains in strength in the non-operative quadriceps—important for long-term ability to normalize function, and to accelerated recovery of functional mobility (stair descent). These data are all derived from older adults. Preserving quadriceps strength is clinically important because poor strength is directly linked to reduced physical function and gait. Quadriceps weakness inhibits balance (84), reduces functional mobility (20, 82), and increases the risk of falls (83) in older adults. Our preliminary data suggest that patients on EAA treatment lost less muscle from their extremities compared to subjects on placebo. An additional potential benefit will be our potential to provide physical therapy to patients without insurance or in instances where the subjects insurance does not pay for the total number of visits we require for this study. These may be considered a potential benefit.

We repeated this study with 39 patients in 2014-2016 and again found the group taking EAA lost less muscle than the placebo group.

Investigator Experience

University of Oregon

Hans C. Dreyer, PT, PhD. (3.0 calendar months, 25% effort). Role: Principal Investigator. Dr. Dreyer is an expert in translational research on molecular muscle biology and measurements of human muscle metabolism in aging. He has received significant training as a post-doctoral fellow, K12 Research Scholar, and Mentored Research Scientist Development Awardee (K01), and is now at the end of his 5-year K01 award, which seeks to determine the potential for amino acid supplementation to augment return of muscle metabolism and physical function in older adults during rehabilitation from total knee replacement surgery. He will assume overall responsibility for the management of the study, has assign responsibilities for the other members of the team, will ensure that informed consent is properly obtained from prospective patients, be the liaison to the Institutional Review Boards (IRB), oversight plan (DSMP), report adverse events and in discussions with the physicians determine if AE are study related or not, and ensure the accuracy of the data. As well, Dr. Dreyer will be responsible for subject recruitment, experimental procedures, tracer study supervision, tissue/data collection, data/tissue processing, data analysis, manuscript preparation, draft finalization, and annual reports to the NIH and ClinicalTrials.gov. Dr. Dreyer will consult via teleconference with Dr. Kuehl on a regular bi-weekly basis for the first six months of the study. Thereafter, they will teleconference once a month until study completion. Dr. Dreyer and Dr. Kuehl will be responsible for preparing annual and final report as well as post-enrollment activities. Dr. Dreyer will have direct contact with subjects and will have contact with identifiable and non-identifiable subject data.

Anita Christie, PhD. Aim 1 (1.2 calendar months, 10% effort). Role: Co-Investigator. Dr. Christie is an expert in research on neuromuscular changes with advanced age in humans. She has received extensive training as a doctoral student and post-doctoral fellow in measuring both electrically- and magnetically-evoked muscle potentials to assess nervous system (central) and muscle (peripheral) function. She also has extensive experience in the processing and interpretation of surface electromyographic signals. Dr. Christie and her lab assistants will be responsible for obtaining, processing, and interpreting measures of central activation deficits (CAD) of all subjects at Baseline and at 6 wks (Aim 1) and 6 mo and 1 yr (Aim 2). Dr. Christie will meet with Dr. Dreyer on a regular basis to discuss data analysis and to provide technical expertise related to the effects of surgery on activation deficits. Dr. Christie's research experience and publication record make her a valuable member of this research project. Dr. Christie's office is on the 3rd floor of the Center for Medical Education and Research, the same building where Dr.

Dreyer's office is located. Dr. Christie will assist with data collection, data/tissue processing, data analysis, and manuscript preparation, and will present findings at national conferences. Dr. Christie will meet with the entire research team once a month to discuss issues related to the project. Dr. Christie will have direct contact with subjects and will have contact with non-identifiable subject data.

Carry E. McCurdy, PhD. (<1 calendar months, 3% effort). Role: Co-Investigator. Dr. McCurdy is an expert in integrative physiology. She has received significant training as a post-doctoral fellow and Instructor. Dr. McCurdy is experienced in conducting mechanistic animal and cell culture experiments that are closely aligned with the proposed project. Dr. McCurdy will meet regularly with Dr. Dreyer to discuss data analysis, and to provide technical expertise related to the design, implementation and oversight of the cell culture studies and will, along with Dr. Dreyer, supervise the training of graduate students and technicians in methods of in vitro experimentation using cell culture. Dr. McCurdy will be responsible for oversight of the cell culture studies and will, along with Dr. Dreyer, supervise the training of graduate students and technicians in methods of in vitro experimentation using cell culture. Dr. McCurdy has a strong record of research and publications, and will be a valuable member of the investigative team. Dr. McCurdy will not have direct contact with subjects or subject data.

University Research Assistants

Jonathan Muyskens, PhD. (12 calendar months, 100% effort). Role: Research Assistant. Perform human functional testing, strength determination, help with biopsies, MRI analysis, MRI scan buddy, and biochemistry (blood and tissue analysis).

Slocum Education and Research Foundation

Brian A. Jewett, MD (<1 calendar months, 3% effort). Role: Co-Investigator. Dr. Jewett is an orthopedic surgeon at the Slocum Center for Orthopedics & Sports Medicine. Dr. Jewett specializes in adult joint reconstruction. A Diplomate of the American Board of Orthopedic Surgery since 2003, Dr. Jewett has collaborated with Dr. Dreyer on projects investigating the beneficial effects of using essential amino acid supplementation to boost recovery following TKA surgery since 2009. Dr. Jewett will facilitate screening and recruitment of potential subjects into the study. Dr. Jewett and Dr. Dreyer, along with the other Co-Investigator physicians, the study coordinator, and Erin Owen, MPH, the Director of the Slocum Education and Research Foundation, will meet regularly to discuss recruitment, enrollment, and retention profiles as well as any issues related to adverse events. Dr. Jewett's office is housed in the Slocum Center for Orthopedics & Sports Medicine, which is located 0.5 miles from Dr. Dreyer's laboratory and offices in the Center for Medical Education and Research. Dr. Jewett and Dr. Dreyer meet regularly each month for research meetings. Dr. Jewett will have direct contact with subjects and subject data, which is a component of his position as orthopedic surgeon.

Brick A. Lantz, MD. (<1 calendar months, 3% effort). Role: Co-Investigator. Dr. Lantz is an orthopedic surgeon at the Slocum Center for Orthopedics & Sports Medicine. Dr. Lantz also serves as President of Slocum Research & Education Foundation, a 501(c) 3 organization dedicated to clinical research, outcomes research, and physician education projects. Dr. Lantz specializes in TKA, osteotomy, and treatment of lower extremity trauma. Dr. Lantz will facilitate screening and recruitment of potential subjects into the study. Dr. Lantz and Dr. Dreyer have collaborated on projects involving TKA patients

since 2009. Dr. Lantz and Dr. Dreyer, along with the other Co-Investigator physicians, the study coordinator, and Erin Owen, MPH, the Director of the Slocum Education and Research Foundation, will meet regularly to discuss recruitment, enrollment, and retention profiles as well as any issues related to adverse events. Dr. Lantz's office is housed in the Slocum Center for Orthopedics & Sports Medicine, which is located 0.5 miles from Dr. Dreyer's laboratory and offices in the Center for Medical Education and Research. Dr. Lantz and Dr. Dreyer meet regularly each month for research meetings. Dr. Lantz will have direct contact with subjects and will have contact with non-identifiable subject data, which is a component of his position as orthopedic surgeon.

Craig G. Mohler, MD. (<1 calendar months, 3% effort). Role: Co-Investigator. Dr. Mohler is an orthopedic surgeon at the Slocum Center for Orthopedics & Sports Medicine. Dr. Mohler specializes in TKA, osteotomy, and treatment of lower extremity trauma. Dr. Mohler will facilitate screening and recruitment of potential subjects into the study. Dr. Mohler and Dr. Dreyer, along with the other Co-Investigator physicians, the study coordinator, and Erin Owen, MPH, the Director of the Slocum Education and Research Foundation, will meet regularly to discuss recruitment, enrollment, and retention profiles as well as any issues related to adverse events. Dr. Mohler's office is housed in the Slocum Center for Orthopedics & Sports Medicine, which is located 0.5 miles from Dr. Dreyer's laboratory and offices in the Center for Medical Education and Research. Dr. Mohler will have direct contact with subjects and will have contact with non-identifiable subject data, which is a component of his position as orthopedic surgeon.

Steven N. Shah, MD. (<1 calendar months, 3% effort). Role: Co-Investigator. Dr. Shah is an orthopedic surgeon at the Slocum Center for Orthopedics & Sports Medicine. Dr. Shah specializes in primary and revision hip and knee replacement surgeries. His research interests include the treatment of hip and knee arthroplasty. Dr. Shah will facilitate screening and recruitment of potential subjects into the study. Dr. Shah and Dr. Dreyer have collaborated on projects involving TKA patients since 2010. Dr. Shah and Dr. Dreyer, along with the other Co-Investigator physicians, the study coordinator, and Erin Owen, MPH, the Director of the Slocum Education and Research Foundation, will meet regularly to discuss recruitment, enrollment, and retention profiles as well as any issues related to adverse events. Dr. Shah's office is housed in the Slocum Center for Orthopedics & Sports Medicine, which is 0.5 miles from Dr. Dreyer's laboratory and offices in the Center for Medical Education and Research. Dr. Shah and Dr. Dreyer meet regularly each month for research meetings.
Dr. Shah will have direct contact with subjects and will have contact with non-identifiable subject data, which is a component of his position as orthopedic surgeon.

Julie Embree, PT. Aim1 (1.0 calendar months, 8% effort). Role: Consultant. Ms. Embree is the Director of the Slocum Therapy Center. Her role in the proposed research, along with the PI, will be to establish standardized outpatient physical therapy at her site as well as at all local and distant sites per patient preference and insurance coverage. She will have bi-weekly meetings with Dr. Dreyer and Erin Owen, MPH, Director of the Slocum Education and Research Foundation, and our study coordinator, as well as phone/email contact as needed—in the proposed study. Ms. Embree's office is on first floor of the Slocum Center for Orthopedics & Sports Medicine. Ms. Embree will communicate regularly with Dr. Dreyer regarding patient therapy. Ms. Embree will meet twice weekly with the study coordinator to discuss newly enrolled participant therapy center and facilitate recording/standardization of therapy at each location.
Ms. Embree will have direct contact with subjects and will have contact with non-identifiable subject data, which is a component of her position at Slocum Physical Therapy.

Study Coordinators

Erin Owen, Tessa Kirkpatrick & Michelle Bremer(12 calendar months, 100% effort). Role: Study Coordinator. The study coordinators will be responsible for coordinating all patient centered activity beginning with ensuring Slocum staff pre-identify TKA patients based on inclusion/exclusion criteria prior to clinic visits. The study coordinators and the surgeon will have a list of all potentially eligible subjects having appointments with Drs. Jewett, Lantz, Mohler, and Shah prior to arriving for clinic visits. Once entry criteria are verified by the surgeon, the study coordinator will explain the study, provide an informed consent and follow up with patients by phone. The study coordinator, along with Dr. Dreyer and Drs. Jewett, Lantz. Mohler, and Shah, will be responsible for ensuring enrollment be maintained at 3+ subjects/month. The study coordinator will ensure data collection and management coordination with Dr. Dreyer and Dr. Smolkowski necessary for periodic and annual reports.

Study coordinators of the Slocum Education and Research Foundation have office space located on the first floor of the Slocum Center for Orthopedics and Sports Medicine. Coordination through the Slocum Education and Research Foundation will allow for seamless integration with existing Slocum infrastructure with all the necessary credentialing and security clearances. The study coordinators have experience with clinical research. As such, the study coordinators will be responsible for the coordination of the study participants and retention via frequent phone and in person visits. The study coordinator and Dr. Dreyer will discuss subject enrollment goals on a day to day basis and meet biweekly. The study coordinators will be on-site and work with Slocum staff to maximize pre-screening, recruitment, enrollment, and retention of the 120 subjects over the 5 years of the proposed study. Each month, the Study Coordinator and Dr. Dreyer will meet with the full research team to provide study updates, to discuss all aspects of the project, and to ensure that the research is meeting its goals and objectives. Significant effort will be dedicated to subject enrollment and phone/email/in person follow-up to ensure that we meet our targeted enrollment each month. Coordinators will have direct contact with subjects and will have contact with non-identifiable subject data, which is a component of her position at Slocum.

Oregon Research Institute

Biostatisticians

Keith Smolkowski, PhD. [(1.2 calendar months, 10% effort)]. Role: [Co-Investigator.] Dr. Smolkowski is a Statistician with Oregon Research Institute, Eugene, OR. He will oversee statistical analysis and modeling in the proposed study. Dr. Smolkowski's office is located less than 1 mile from Dr. Dreyer's office. Dr. Smolkowski is currently collaborating with Dr. Dreyer and has frequently run statistical analysis for researchers from the Department of Human Physiology and the Department of Education at the University of Oregon. Dr. Smolkowski and his statistical team will meet with the research team once a month or more, as needed. Advanced statistical modeling will be employed to ensure we have the necessary power to correlate change in physical function with cellular data obtained from biopsies, blood samples, and imaging. Because we propose a longitudinal clinical trial which has the potential for missing data points, Dr. Smolkowski's statistical analyses will use modeling methods to account for potential missing data points, further assuring that we will accomplish our aims within the 5-year study period. Consultation with Dr. Smolkowski is necessary for meeting both Aims of this proposal. Dr. Smolkowski will not have direct contact with subjects but will have contact with non-identifiable subject data.

Lisa Strycker, MA. (Consultant; no salary support requested). Role: Consultant. Ms. Strycker is a Senior Research Associate with Oregon Research Institute, Eugene. In the proposed investigation, she will be responsible for all data management and will assist Dr. Smolkowski with statistical analysis. She has office space adjacent to Dr. Smolkowski. She is currently collaborating with Dr. Dreyer, has analyzed all of the preliminary data for this grant proposal, and has more than 20 years of substantive and methodological experience in conducting health-related clinical trials involving adults, older women, chronic illness, physical activity, longitudinal designs, data imputation, and other research areas. She coauthored "An Introduction to Latent Variable Growth Curve Modeling" as part of Lawrence Erlbaum Associates' Quantitative Methodology Series, which focuses on specialized analytic techniques for longitudinal data and mixed methods. For the proposed study, Ms. Strycker will update, clean, and manage the large volume of data collected in real time from the Study Coordinator; will be blinded to patient randomization; will routinely meet and provide data reports to the investigative team; and will help Dr. Smolkowski and the project investigators plan and conduct data analyses to answer the research questions and meet the Specific Aims. Ms. Strycker will not have direct contact with subjects but will have contact with non-identifiable subject data.

Oregon Health and Sciences University

Kerry Kuehl, MD, DrPH. (<1 calendar months, 5% effort). Role: Co-Investigator. Kerry S. Kuehl, MD, DrPH, is Professor of Medicine and Co-Director of the Human Performance Laboratory in the Division of Health Promotion & Sports Medicine at OHSU. He is a primary care physician and clinical researcher specializing in health promotion and disease prevention strategies focusing on economic outcomes. Dr. Kuehl is the P.I. of the SHIELD Study assessing the effects of a worksite wellness program on police officers safety and health outcomes. He is a PHLAME Study co-investigator, and performed a retrospective analysis of workers' compensation claims and reportable injuries among PHLAME and non-PHLAME fire fighters finding a significant reduction in injury rates and costs among PHLAME departments. Dr. Kuehl received a prestigious K23 Career Development Training grant by the National Institutes of Health in 2003 and was the Principal Investigator of the CHOICE (Changing Healthy Outcomes In Clinical Environment) Study. Dr. Kuehl is an expert in clinical nutrition and sport supplements. As a Co-Investigator Dr. Kuehl will collaborate and provide expert advise on the oversight and administration of the clinical trial. Dr. Kuehl will email and teleconference with Dr. Dreyer on a bi-weekly basis to discuss management issues and strategies to mitigate events that may arise during the course of the study. Once a month Dr. Kuehl will participate in a conference call with all key personnel on this project, organized by Dr. Dreyer. Dr. Kuehl will meet in person with Dr. Dreyer and key personnel twice yearly to discuss the progress of the trial and subject matriculation and recruitment goals/milestones. Dr. Kuehl will teleconference with the entire research team once a month for the duration of the study. Dr. Kuehl and Dr. Dreyer will be responsible for preparing annual and final report. Dr. Kuehl will not have direct contact with subjects or subject data.

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Appendix 1: Data Specifications Table

Data elements required to meet study specific aims come from multiple sources: patient self-report, electronic health records or administrative data, study-specific questionnaires and surveys, functional tests and assessments, and results of study-specific procedures. The tables below describe each data element and intended use. The study coordinators at the Slocum Foundation will abstract data from clinic and hospital electronic health records, and when required, capture data by patient-self report.

Data Elements Table. Data specifications for hospitals and practice administrative data, hospital or practice electronic medical records, and/or client self-report.

Data Element ID	Data Element	Type	Description and Intended Use	Specific Aim #
1	Client ID number	Numeric	Unique person identifier.	1,2
2	Date of Birth	Date	Calculated age at study-specific time points; data analysis, stratification, independent covariate.	1,2
3	Race	String	Data analysis, stratification, independent covariate.	1,2
4	Ethnicity	String	Data analysis, stratification, independent covariate.	1,2
5	Height	Numeric	Data analysis, stratification, independent covariate.	1,2
6	Weight	Numeric	Data analysis, stratification, independent covariate.	1,2
7	BMI	Calculated	Data analysis, stratification, independent covariate.	1,2
8	Tourniquet up	Time	Data analysis, stratification, independent covariate.	1,2
9	Tourniquet down	Time	Data analysis, stratification, independent covariate.	1,2
10	Operating Room Time Start (In room)	Time	Data analysis, stratification, independent covariate.	1,2
11	Procedure Start	Time	Data analysis, stratification, independent covariate.	1,2
12	Procedure Stop	Time	Data analysis, stratification, independent covariate.	1,2
13	Operating Room Stop Time (Out of room)	Time	Data analysis, stratification, independent covariate.	1,2
14	Date of Admit	Date	Calculation of Length of Stay; Data analysis, stratification, independent covariate.	1,2
15	Date of Discharge	Date	Calculation of Length of Stay; Data analysis, stratification, independent covariate.	1,2

Data Elements Table. Data specifications for hospitals and practice administrative data, hospital or practice electronic medical records, and/or client self-report (continued).

Data Element ID	Data Element	Type	Description and Intended Use	Specific Aim #
16	Medication Name 1 – x	String	Data analysis, stratification, independent covariate.	1,2
17	Medication Dose (1 – x)	Numeric	Data analysis, stratification, independent covariate.	1,2
18	Medication Route (1 – x)	String	Data analysis, stratification, independent covariate.	1,2
19	Medication Frequency (1 – x)	String	Data analysis, stratification, independent covariate.	1,2
20	Medication Start Date (1 – x)	Date	Data analysis, stratification, independent covariate.	1,2
21	Medication Stop Date (1 – x)	Date	Data analysis, stratification, independent covariate.	1,2
22	Health Condition (1 – x)	String	Data analysis, stratification, independent covariate.	1,2
23	Health Condition Start Date (1 – x)	Date	Data analysis, stratification, independent covariate.	1,2
24	Health Condition Stop Date (1 – x)	Date	Data analysis, stratification, independent covariate.	1,2
25	Additional procedures (1 – x)	String	Any additional medical procedures or tests performed while patient is on the study protocol; Data analysis, stratification, independent covariate.	1,2
26	Tobacco Use	Categorical	Data analysis, stratification, independent covariate.	1,2
27	Tobacco Use Frequency	Numeric	Cigarettes/day; Data analysis, stratification, independent covariate.	1,2
28	Alcohol Use	Categorical	Data analysis, stratification, independent covariate.	1,2
29	Alcohol Use Amount	Numeric	Drinks/Day of beer, wine or liquor; Data analysis, stratification, independent covariate.	1,2
30	Exercise Frequency	Categorical	Data analysis, stratification, independent covariate.	1,2
31	Charlney Classification of Disease	Categorical	Degree of disability or disease in operative and non-operative knee; Data analysis, stratification, independent covariate.	1,2

Data Elements Table. Data specifications for hospitals and practice administrative data, hospital or practice electronic medical records, and/or client self-report.				
Data Element ID	**Data Element**	**Type**	**Description and Intended Use**	**Specific Aim #**
32	Physical Therapy Utilization	Count	Data analysis, stratification, independent covariate.	1,2
33	Readmission	Dichotomous (Yes/No)	Data analysis, stratification, independent covariate.	1,2
34	Reoperation	Dichotomous (Yes/No)	Data analysis, stratification, independent covariate.	1,2
35	Clinic Visit Utilization	Count	Data analysis, stratification, independent covariate.	1,2
36	Performing Surgeon ID	Numeric	Known confounder in TKA outcome studies; Data analysis, stratification, independent covariate.	1,2
37	Discharge Disposition	Categorical	Patient discharged to home, rehabilitation or skilled nursing; potential confounder; data analysis, stratification, independent covariate.	1,2
38	Hemoglobin (pre-admit through day of discharge)	Numeric	Data analysis, stratification, independent covariate.	1,2
39	Blood Transfusions	Dichotomous (Yes/No)	Data analysis, stratification, independent covariate.	1,2
40	Count of Transfusions (units)	Count	Data analysis, stratification, independent covariate.	1,2
41	Study-specific Laboratory Assessment*	Numeric	Patient safety; data analysis, stratification, independent covariate.	1
42	Blood Analysis Standard of Care and Study-specific Laboratory Assessment*	Numeric	FDA requested monitoring of EAA use, patient safety; data analysis, stratification, independent covariate.	1
43	Menopausal status	Categorical	DSMB recommendation; potential confounder of primary endpoints.	1,2
**Please refer to the protocol for study-specific lab assessments.*				

Data Elements Table. Data specifications for data collected through patient questionnaires or surveys.

Data Element	Survey or Questionnaire Name	Research Specific or Standard of Care	Description and Intended Use	Specific Aim #
1	VR-12	Standard of Care*	Health-related quality of life; Data analysis, stratification, independent covariate.	1,2
2	Patient Activation Measure (PAM)	Research	Patient knowledge and confidence managing health conditions; Data analysis, stratification, independent covariate.	1,2
3	Oxford Knee Score	Standard of Care*	Knee-specific functional assessment; Data analysis, stratification, independent covariate.	1,2
4	Knee Injury and Osteoarthritis Outcome Score (KOOS)	Research	Knee-specific functional assessment; Data analysis, stratification, independent covariate.	1,2
5	Pain Scale or Visual Analogue Pain Scale	Standard of Care*	Pain assessments; Data analysis, stratification, independent covariate.	1,2
6	Patient Health Questionnaire-9 Item (PHQ-9)	Research	Screening for depression and documenting severity of depression (known confounder in TKA outcome studies); Data analysis, stratification, independent covariate.	1,2
7	Serious Life Events	Research	Identification of serious life events that may impact key study endpoints; Data analysis, stratification, independent covariate.	1,2
8	The Pain Catastrophizing Scale (PCS)	Research	Types of thoughts and feelings experienced with pain; data analysis, stratification, independent covariate.	2

**Data transmitted securely to study team by Slocum Orthopedics. Standard of care instruments are collected by electronic data capture on iPads.*

Data Elements Table. Data specifications for data collected through functional tests and study-specific assessments.				
Data Element	**Test, Assessment, or Procedure**	**Collection Method**	**Description and Intended Use**	**Specific Aim #**
1	Food Log/Diaries	Patient self-report	Caloric intake, stratified by protein, fat, carbohydrate and total calories; Data analysis, stratification, independent covariate.	1
2	Accelerometer	Download of accelerometer data	Patient mobility; Data analysis, stratification, independent covariate.	1
3	Get Up and Go Test	Study staff assessment	Stand from sitting and walk; standardized functional test; study endpoint.	1,2
4	6-minute Walk	Study staff assessment	Standardized functional test; study endpoint.	1,2
5	Stair Climb Up	Study staff assessment	Standardized functional test; study endpoint.	1,2
6	Stair Climb Down	Study staff assessment	Standardized functional test; study endpoint.	1,2
7	Grip Strength – Hand	Study staff assessment	Standardized functional test; study endpoint.	1,2
8	4 meter walk	Study staff assessment	Standardized functional test; study endpoint.	1,2
9	Standing Balance Test	Study staff assessment	Standardized functional test; study endpoint.	1,2
10	Chair Stand Test (Sit to stand five times)	Study staff assessment	Standardized functional test; study endpoint.	1,2
11	MRI; Leg Composition Measurements	Study staff assessment	Bilateral assessment of leg composition; study endpoint.	1
13	Isometric Extensor	Study staff assessment	Bilateral assessment of quadriceps strength; study endpoint.	1
14	Isometric Flexor	Study staff assessment	Bilateral assessment of hamstring strength; study endpoint.	1
15	Essential Amino Acid or Placebo Supplementation Log	Patient self-report	Compliance with study protocol; study intervention.	1,2
16	Muscle & Biopsy	Study staff	Bilateral biopsy; study endpoint(s).	1,2
17	Edema & Fluid Accumulation	Study staff	Bilateral biopsies; controlling for potential confounding of study endpoints.	1,2
#Please refer to the protocol for study-specific lab measurements.				

Appendix 2: Study Logs

Medical Condition Log

To be updated by study staff with patient

Condition Name or Description	Date of Onset	Date Resolved	Check if Ongoing
			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
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			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
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			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
			☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6

IRB Reviewer Note: For Aim #2, the study timeline check boxes will updated.

Page _______ of _________

Medication Log

To be updated by study staff with patient

Medication Name or Description	Route	Frequency	Dose	Check if Ongoing
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6
	☐ oral ☐ nasal ☐ IM ☐ IV ☐ Sub-q	☐ prn ☐ qd ☐ bid ☐ tid ☐ ________		☐ - 6 week ☐ week 1 ☐ - 4 week ☐ week 2 ☐ - 1 week ☐ week 6

IRB Reviewer Note: For Aim #2, the study timeline check boxes will updated.

Page _________ of __________

Study Medication – Essential Amino Acid or Placebo Log
Week _____

Date	Day of the Week	Morning Dose Time	Physical Therapy Time or No PT Today (check)	Afternoon Dose Time
01/01/2014	***Monday***	***10:00a***	☐ No PT Today ***2:30p***	***3:30p***
			☐ No PT Today	
			☐ No PT Today	
			☐ No PT Today	
			☐ No PT Today	
			☐ No PT Today	
			☐ No PT Today	
			☐ No PT Today	

Patient Comments:

***Instructions*:** Each day at approximately 10 AM (but at least two hours after you have eaten breakfast) you will to mix one bottle of pre-weighed supplement into something of your choosing (juice or yogurt, for example) and ingest/drink. You will do this again approximately 2 hours after lunch (~2 PM). If you miss a time point you can make up that time point later in the day however we request that you do so at least 3 to 4 hours apart (supplements in a day) and that you record the time on your log.

On those occasions when you receive physical therapy treatment you will ingest the supplement within one hour after treatment. If you have treatment by physical therapy in the morning that will be your morning supplement for the day and your next supplement will be at approximately 2 PM. If you have physical therapy in the afternoon, your afternoon (second supplement of the day) will be ingested within one hour after therapy. *This is very important as our research has shown that supplement ingestion is much better for you if taken within one hour of exercise.*

If you have questions about your study medication, please call your research coordinators 541.868.3232.

Pre-Operative Heavy Water Log
Week _____

Date	Morning Time	Mid-Afternoon Time	Evening Time	Patient Comments
01/01/2014	***10:00a***	***2:00p***	***7:00p***	***No side effects.***

Patient Comments:

***Instructions*:** In order to measure muscle metabolism you will also be asked to ingest tracer water (heavy water).The tracer water contains something called an isotope. An isotope is a normal substance that contains an unusual number of neutrons (very, very small particles found in all things). The isotope is usually present in normal water but the tracer water you will drink will contain more than usual. We want you to drink the tracer water so that we can measure reactions in the body. You will not notice/taste the isotope, nor will it change anything in your body. Each day at approximately 10 AM, 2 PM, and 7 PM, you will ingest 50 ml (1.7 ounces) of tracer water. You will also be allowed to drink normal water. Overall, 95% of people experience no side-effects from drinking heavy water. *We want to use this heavy water to measure how your cells respond to supplement before surgery.*

If you have questions about taking heavy water, please call your research coordinators, Erin or Tessa at 541.868.3232.

Post-Operative Heavy Water Log
Week _____

Date	Morning Time		Evening Time	Patient Comments
01/01/2014	***10:00a***		***7:00p***	***No side effects.***

Patient Comments:

***Instructions*:** Just like before you had surgery you will again ingest tracer water (heavy water). Each day at approximately 10 AM and 2 PM, you will ingest 50 ml (1.7 ounces) of tracer water. You will continue to ingest the heavy water for one or two weeks after surgery, depending on randomization. You will also be allowed to drink normal water. *We want to use this heavy water to measure how your cells respond to supplement after surgery.*

You are randomized to drink heavy water for _____week(s) after surgery.

If you have questions about taking heavy water, please call your research coordinators, Erin or Tessa at 541.868.3232.

Accelerometer Log

To be updated by study staff

Date	Location	Device Number Distributed (or N/A)	Device Number Returned (or N/A)	Coordinator Comments/Notes	Coordinator Initials

Page _____ of _____

Patient Phone or Contact Log

To be updated by study staff

Date	Location	Notes

Page _____ of _____

Patient Physical Therapy Appointment Tracking Log
Heavy Water Study

To be updated by patient

Appt Number	Date	Location	Notes
1			Post-Operative Day #5
2			Post-Operative Day #7
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			

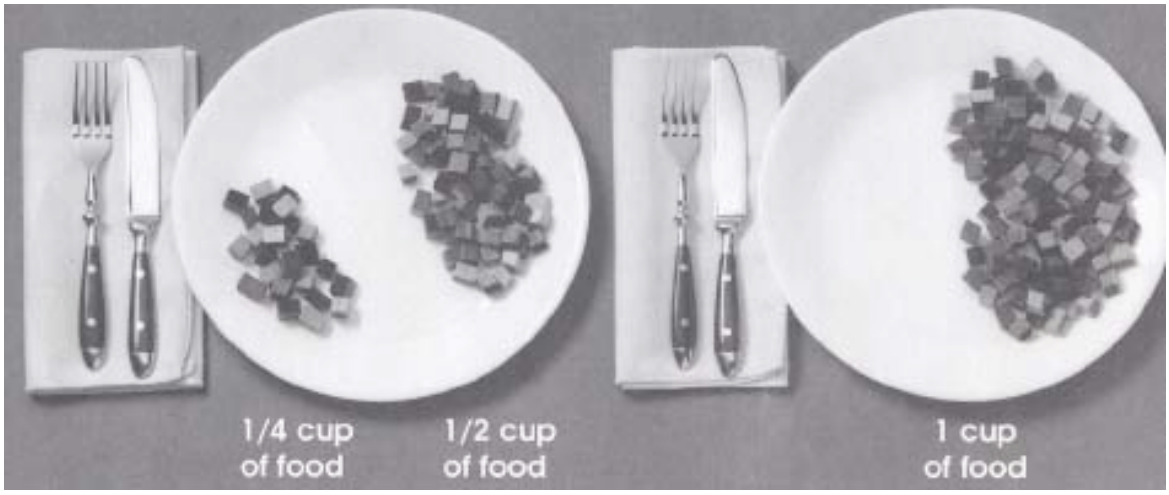
Page _____ of _____

Instructions for Keeping 3 Days of Food Records

1. Keep a careful and accurate record of all FOOD and beverages (including coffee, water, gum, etc.) consumed during 3 days.
2. Try to write down food as you eat it. Do not rely on your memory.
3. Estimate the amounts in terms of household measures (like 1 cup, or 8 oz. milk, 3 oz. fish, 1 teaspoon butter). 1 pat of butter is about 1 teaspoon. (See sample picture on next page.)
4. 8 ounces = 1 cup ONLY when the item is FLUID. Do not use ounces unless the food is liquid (8 oz. milk) or you know what it weighs (3 oz. chicken). 8 oz. Cheerios does NOT equal 1 cup. 8 oz. Cheerios is half the box.
5. Use the label and package when possible to write down specific amounts eaten. For example, if you eat a whole bag of chips, you can find the oz. wt. on the package. Make sure you look at the servings per package, and calculate accordingly. Often times, a bag of chips might have 2.5 oz. servings, so if you eat the whole package you would take that into account.
6. 1 ounce cheese = 1 slice cheese = 1/4 cup shredded cheese.
7. 3 oz. of meat is about the size of a deck of cards.
8. Note the food's preparation method (baked, fried, etc.).
9. Be specific about the type of milk (whole, 2%, 1%, nonfat).
10. Don't forget the spreads on bread, water, salad dressings, gravies, cream and sugar in coffee, etc.
11. For mixed dishes, like casseroles, estimate and record the amounts of the major ingredients.
12. For fast food restaurants, record the name of the restaurant.

Please call Erin Owen or Tessa Kirkpatrick at 541.868.3232 if you have any questions about your food diary.

A visual for estimating your portions.



Food Diary Day 1

Day of the week: _____

Date: _____

Your initials: _____

1. BREAKFAST *(time: _____)*		**4. SNACK *(time: _____)***	
Amount	**Food**	**Amount**	**Food**
		5. DINNER *(time: _____)*	
		Amount	**Food**
2. SNACK *(time: _____)*			
Amount	**Food**		
3. LUNCH *(time: _____)*			
Amount	**Food**		
		6. SNACK *(time: _____)*	
		Amount	**Food**

Food Diary Day 2

Day of the week: _____

Date: _____

Your initials: _____

1. BREAKFAST *(time: _____)*		**4. SNACK *(time: _____)***	
Amount	**Food**	**Amount**	**Food**
		5. DINNER *(time: _____)*	
		Amount	**Food**
2. SNACK *(time: _____)*			
Amount	**Food**		
3. LUNCH *(time: _____)*			
Amount	**Food**		
		6. SNACK *(time: _____)*	
		Amount	**Food**

Food Diary Day 3

Day of the week: _____
Date: _____
Your initials: _____

1. BREAKFAST *(time: _____)*		**4. SNACK *(time: _____)***	
Amount	**Food**	**Amount**	**Food**
		5. DINNER *(time: _____)*	
		Amount	**Food**
2. SNACK *(time: _____)*			
Amount	**Food**		
3. LUNCH *(time: _____)*			
Amount	**Food**		
		6. SNACK *(time: _____)*	
		Amount	**Food**

Adverse Event Report

To be updated by study staff

Subject Adverse Event Number: _____

Description of AE or Diagnosis:

_____

Study Relationship:
☐ Not related ☐ Possibly related ☐ Probably related ☐ Definitely related

Severity Grading: ☐ Mild ☐ Moderate ☐ Severe ☐ Life Threatening

Involves operative site/knee? ☐ Yes ☐ No

Outcome:	Date of outcome or outcome change:	Physician Investigator initials & date	Coordinator initials & date when study AE log is updated
Ongoing			
Resolved, residual effects present and being treated			
Death			
Resolved, No sequel			
Unknown			

Action Taken Regarding Study Intervention:
- ☐ None
- ☐ Discontinued Permanently
- ☐ Discontinued Temporarily
- ☐ Reduced Dose
- ☐ Delayed Dose

Other Action Taken: ☐ None
_____
_____
_____

Did AE result in hospital admission (outpatient or inpatient)? ☐ Yes ☐ No → If yes, readmission date: _____

Was the event serious? ☐ Yes ☐ No → If yes, under what criteria (check below)?
- ☐ Death
- ☐ Life-Threatening
- ☐ Hospitalization (initial or prolonged)
- ☐ Disability or permanent damage
- ☐ Required intervention to prevent permanent impairment
- → *Requires immediate reporting to IRB and DSMB if serious. Report date:* _____

Physician Investigator Signature: _____ **Date:** _____

IRB Reviewer Note: For Aim #2, the study timeline check boxes will updated.

Protocol Deviation or Violation Report

To be updated by study staff

Protocol Deviation Report Number: _____

Description of Event:

_____

Study Interval:

- ☐ - 6 weeks
- ☐ - 4 weeks
- ☐ - 1 week
- ☐ Day of Surgery through Discharge
- ☐ 1 week
- ☐ 2 week
- ☐ 6 week

Type of Deviation:

- ☐ Missed study activity
- ☐ Out of window
- ☐ Patient refused
- ☐ Investigator judgment or decision

Result of Deviation:

- ☐ Subject withdrew from study participation
- ☐ Investigator withdraws subject from study participation
- ☐ Subject continues enrollment and participation per protocol
- ☐ Other: _____

Did this event increase the risk or decrease the benefit, affect subject's rights, safety or welfare, or integrity of the data? ☐ Yes ☐ No → *If yes, report protocol violation to the IRB and DSMB.*

Date reported: _____ By: _____

PI Signature: _____ **Date:** _____

Physician Investigator Signature: _____ **Date:** _____

IRB Reviewer Note: For Aim #2, the study timeline check boxes will updated.

Appendix 3: Patient-Reported Questionnaires

Social Readjustments Rating Scale – Life Events Questionnaire (Administered on paper)

Give to patient to fill out themselves or read through with patient. To measure stress according to the Holmes and Rahe Stress Scale, the number of "Life Change Units" that apply to events in the past year of an individual's life are added and the final score will give a rough estimate of how stress affects health.

Life Event	Life Change Units
Death of a spouse	100
Divorce	73
Marital separation	65
Imprisonment	63
Death of a close family member	63
Personal injury or illness	53
Dismissal from work	47
Marital reconciliation	45
Retirement	45
Change in health of family member	44
Pregnancy	40
Gain a new family member	39
Business readjustment	39
Change in financial state	38
Death of a close friend	37
Change to different line of work	36
Change in frequency of arguments	35
Major mortgage	32
Foreclosure of mortgage or loan	30
Change in responsibilities at work	29
Child leaving home	29
Trouble with in-laws	29
Outstanding personal achievement	28
Spouse starts or stops work	26
Beginning or end of school	26
Change in living conditions	25
Revision of personal habit	24
Trouble with boss	23
Change in working hours or conditions	20
Change in residence	20
Change in schools	20
Change in recreation	20
Change in church activities	19
Change in social activities	18
Minor mortgage or loan	17
Change in sleep habits	16
Change in number of family reunions	15
Change in eating habits	15
Vacation	13
Christmas	12
Minor violation of law	11

Score above 300 = At risk of illness

Score of 150 – 299 = Risk of illness is moderate (reduced by 30% from above risk)

Score < 150: Only have slight chance of illness

Holmes TH, Rahe RH (1967). "The Social Readjustment Rating Scale". J Psychosom Res 11 (2): 213–8. doi:10.1016/0022-3999(67)90010-4

KOOS KNEE SURVEY (Administered on paper)

Today's date: _____/_____/_____ Date of birth: _____/_____/_____

Name: _____

INSTRUCTIONS: This survey asks for your view about your knee. This information will help us keep track of how you feel about your knee and how well you are able to perform your usual activities. Answer every question by ticking the appropriate box, only one box for each question. If you are unsure about how to answer a question, please give the best answer you can.

Symptoms

These questions should be answered thinking of your knee symptoms during the **last week**.

S1. Do you have swelling in you knee?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S2. Do you feel grinding, hear clicking or any other type of noise when your knee moves?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S3. Does your knee catch or hang up when moving?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S4. Can you straighten your knee fully?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S5. Can you bend your knee fully?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

Stiffness

The following questions concern the amount of joint stiffness you have experienced during the **last week** in your knee. Stiffness is a sensation of restriction or slowness in the ease with which you move your knee joint.

S6. How severe is your knee joint stiffness after first waking in the morning?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

S7. How severe is your knee joint stiffness after sitting, lying or resting **later in the day**?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Pain

P1. How often do you experience pain?

Never	Monthly	Weekly	Daily	Always
☐	☐	☐	☐	☐

What amount of knee pain have you experienced in the last week during the following activities?

P2. Twisting/pivoting on your knee

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P3. Straighten knee fully

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P4. Bending knee fully

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P5. Walking on flat surface

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P6. Going up or down stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P7. At night while in bed

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P8. Sitting or lying

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P9. Standing upright

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Function, daily living

The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

A1. Descending stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A2. Ascending stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

A3. Rising from sitting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A4. Standing

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A5. Bending to floor/pick up an object

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A6. Walking on flat surface

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A7. Getting in/out of car

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A8. Going shopping

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A9. Putting on socks/stockings

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A10. Rising from bed

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A11. Taking off socks/stocking

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A12. Lying in bed (turning over, maintaining knee position)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A13. Getting in/out of bath

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A14. Sitting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A15. Getting on/off toilet

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

A16. Heavy domestic duties (moving heavy boxes, scrubbing floors, etc.)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A17. Light domestic (cooking, dusting, etc.)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Function, sports and recreational activities

The following questions concern your physical function when being active on a higher level. The questions should be answered thinking of what degree of difficulty you have experienced during the **last week** due to your knee.

SP1. Squatting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP2. Running

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP3. Jumping

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP4. Twisting/pivoting on your injured knee

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP5. Kneeling

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Quality of Life

Q1. How often are you aware of your knee problem?

Never	Monthly	Weekly	Daily	Constantly
☐	☐	☐	☐	☐

Q2. Have you modified your life style to avoid potentially damaging activities to your knee?

Not at all	Mildly	Moderately	Severely	Totally
☐	☐	☐	☐	☐

Q3. How much are you troubled with lack of confidence in your knee?

Not at all	Mildly	Moderately	Severely	Extremely
☐	☐	☐	☐	☐

Q4. In general, how much difficulty do you have with your knee?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Thank you very much for completing all the questions in this questionnaire.

PHQ-9 Questionnaire (Administered on paper)

Patient Name _____ **Date** _____

1. Over the last 2 weeks, how often have you been bothered by any of the following problems? Read each item carefully, and circle your response.

	Not at all	Several days	More than half the days	Nearly every day
	0	1	2	3
a. Little interest or pleasure in doing things				
b. Feeling down, depressed, or hopeless				
c. Trouble falling asleep, staying asleep, or sleeping too much				
d. Feeling tired or having little energy				
e. Poor appetite or overeating				
f. Feeling bad about yourself, feeling that you are a failure, or feeling that you have let yourself or your family down				
g. Trouble concentrating on things such as reading the newspaper or watching television				
h. Moving or speaking so slowly that other people could have noticed. Or being so fidgety or restless that you have been moving around a lot more than usual				
i. Thinking that you would be better off dead or that you want to hurt yourself in some way				
Totals				

2. If you checked off any problem on this questionnaire so far, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not Difficult At All	Somewhat Difficult	Very Difficult	Extremely Difficult
0	1	2	3

Oxford Knee Score (Administered Electronically via iPad or on paper)

Please answer the following 12 questions. Choose only one answer per question. The value for each answer is indicated to the right of the answer. Total up all of your answers to obtain a total score out of 48 points. Please only consider how you have been getting on during the past four weeks.

1. How would you describe the pain you have usually from your knee? Score

None – **4**
Very mild – **3**
Mild – **2**
Mild moderate – **1**
Severe – **0**

2. Have you had any trouble with washing and drying yourself all over because of your knee?

No trouble at all – **4**
Very little trouble – **3**
Moderate trouble – **2**
Extreme difficulty – **1**
Impossible to do – **0**

3. Have you had any trouble getting in and out of a car or using public transport because of your knee?

No trouble at all – **4**
Very little trouble – **3**
Moderate trouble – **2**
Extreme difficulty – **1**
Impossible to do – **0**

4. If you were to kneel down could you stand up afterwards?

Yes, easily – **4**
With little difficulty – **3**
With moderate difficulty – **2**
With extreme difficulty – **1**
No, impossible – **0**

5. Have you been limping when walking because of your knee?

Rarely/never – **4**
Sometimes or just at first – **3**
Often, not just at first – **2**
Most of the time – **1**
All of the time – **0**

6. Have you felt that your knee might suddenly give way or let you down?

Rarely/never – **4**
Sometimes or just at first – **3**
Often, not just at first – **2**
Most of the time – **1**
All of the time – **0**

8. Have you been able to do your own household shopping on your own? Score

Yes, easily – **4**
With little difficulty – **3**
With moderate difficulty – **2**
With extreme difficulty – **1**
No, impossible – **0**

9. For how long have you been able to walk before the pain from your knee became severe (with or without a stick)?

No pain, even after more than 30 minutes – **4**
16-30 minutes – **3**
5-15 minutes – **2**
Around the house only – **1**
Unable to walk at all – **0**

10. Have you been able to walk down a flight of stairs

Yes, easily – **4**
With little difficulty – **3**
With moderate difficulty – **2**
With extreme difficulty – **1**
No, impossible – **0**

11. After a meal (sat at a table) how painful has it been for you to stand up from a chair because of your knee?

Not at all painful – **4**
Slightly painful – **3**
Moderately painful – **2**
Very painful – **1**
Unbearable – **0**

12. How much pain from your knee interfered with your usual work (including housework)?

Not at all – **4**
A little bit – **3**
Moderately – **2**
Greatly – **1**
Totally – **0**

13. Have you been troubled by pain from your knee in bed at night?

No nights – **4**
Only 1 or 2 nights – **3**
Some nights – **2**
Most nights – **1**
Every night – **0**

Total Score: /48

THE VETERANS RAND 12 ITEM HEALTH SURVEY (VR-12)

VR-12 (Administered Electronically via iPad or on paper)

Instructions: This questionnaire asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Answer every question by marking the answer as indicated. If you are unsure how to answer a question, please give the best answer you can.

(Circle one number on each line)

1. In general, would you say your health is:

EXCELLENT	VERY GOOD	GOOD	FAIR	POOR
1	2	3	4	5

2. The following questions are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?	YES, LIMITED A LOT	YES, LIMITED A LITTLE	NO, NOT LIMITED AT ALL
a. **Moderate activities,** such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?	1	2	3
b. Climbing **several** flights of stairs?	1	2	3

3. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health?**

	NO, NONE OF THE TIME	YES, A LITTLE OF THE TIME	YES, SOME OF THE TIME	YES, MOST OF THE TIME	YES, ALL OF THE TIME
a. **Accomplished less** than you would like.	1	2	3	4	5
b. Were limited in the **kind** of work or other activities.	1	2	3	4	5

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	NO, NONE OF THE TIME	YES, A LITTLE OF THE TIME	YES, SOME OF THE TIME	YES, MOST OF THE TIME	YES, ALL OF THE TIME
a. **Accomplished less** than you would like.	1	2	3	4	5
b. Didn't do work or other activities as **carefully** as usual.	1	2	3	4	5

5. During the past 4 weeks, how much did **pain** interfere with your normal work (including both work outside the home and house work)?

NOT AT ALL	A LITTLE BIT	MODERATELY	QUITE A BIT	EXTREMELY
1	2	3	4	5

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

6. How much of the time during the past 4 weeks:

	ALL OF THE TIME	MOST OF THE TIME	A GOOD BIT OF THE TIME	SOME OF THE TIME	A LITTLE OF THE TIME	NONE OF THE TIME
a. Have you felt **calm and peaceful**?	1	2	3	4	5	6
b. Did you have **a lot of energy**?	1	2	3	4	5	6
c. Have you felt **downhearted and blue**?	1	2	3	4	5	6

7. During the past 4 weeks, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

ALL OF THE TIME	MOST OF THE TIME	SOME OF THE TIME	A LITTLE OF THE TIME	NONE OF THE TIME
1	2	3	4	5

Now, we'd like to ask you some questions about how your health may have changed.

8. Compared to one year ago, how would you rate your **physical health** in general now?

MUCH BETTER	SLIGHTLY BETTER	ABOUT THE SAME	SLIGHTLY WORSE	MUCH WORSE
1	2	3	4	5

9. Compared to one year ago, how would you rate your **emotional problems** (such as feeling anxious, depressed or irritable) **now**?

MUCH BETTER	SLIGHTLY BETTER	ABOUT THE SAME	SLIGHTLY WORSE	MUCH WORSE
1	2	3	4	5

Patient Activation Measure (Administered Electronically via iPad or on paper)

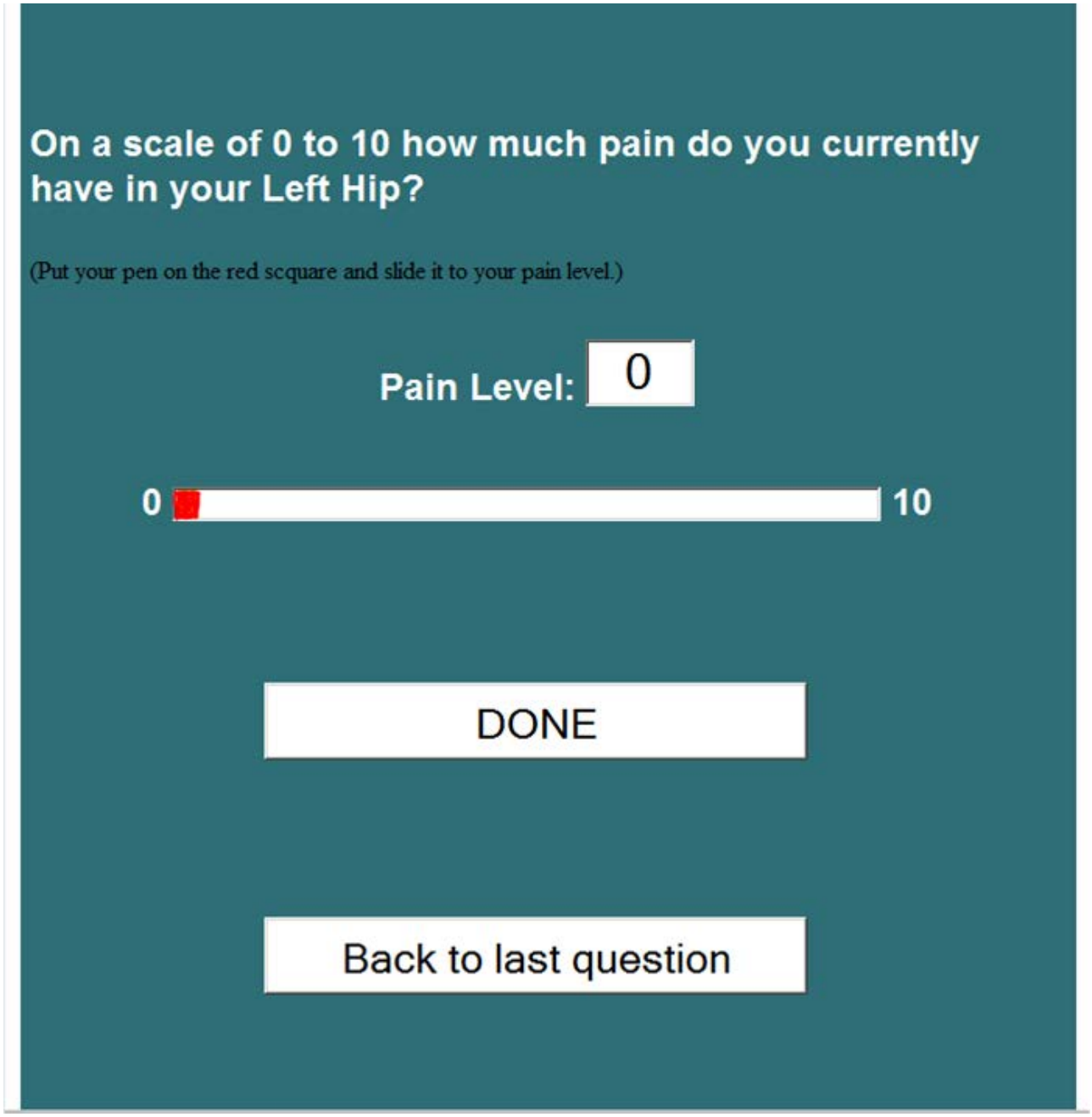
Below are some statements that people sometimes make when they talk about their health. Please indicate how much you agree or disagree with each statement as it applies to you personally by circling your answer. Your answers should be what is true for you and not just what you think others want you to say.

If the statement does not apply to you, circle N/A.

1.	When all is said and done, I am the person who is responsible for taking care of my health	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
2.	Taking an active role in my own health care is the most important thing that affects my health	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
3.	I am confident I can help prevent or reduce problems associated with my health	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
4.	I know what each of my prescribed medications do	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
5.	I am confident that I can tell whether I need to go to the doctor or whether I can take care of a health problem myself	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
6.	I am confident that I can tell a doctor concerns I have even when he or she does not ask	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
7.	I am confident that I can follow through on medical treatments I may need to do at home	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
8.	I understand my health problems and what causes them	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
9.	I know what treatments are available for my health problems	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
10.	I have been able to maintain (keep up with) lifestyle changes, like eating right or exercising	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
11.	I know how to prevent problems with my health	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
12.	I am confident I can figure out solutions when new problems arise with my health	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A
13.	I am confident that I can maintain lifestyle changes, like eating right and exercising, even during times of stress	Disagree Strongly	Disagree	Agree	Agree Strongly	N/A

3

Visual Analogue Pain Scale (Administered Electronically via iPad or on paper)



The Pain Catastrophizing Pain Scale (PCS – administered on paper)

PCS

Client No.: _____ Age: _____ Sex: M(__) F(__) Date: _____

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures or surgery.

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the following scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

0 – not at all **1** – to a slight degree **2** – to a moderate degree **3** – to a great degree **4** – all the time

When I'm in pain …

1. ☐ I worry all the time about whether the pain will end.
2. ☐ I feel I can't go on.
3. ☐ It's terrible and I think it's never going to get any better.
4. ☐ It's awful and I feel that it overwhelms me.
5. ☐ I feel I can't stand it anymore.
6. ☐ I become afraid that the pain will get worse.
7. ☐ I keep thinking of other painful events.
8. ☐ I anxiously want the pain to go away.
9. ☐ I can't seem to keep it out of my mind.
10. ☐ I keep thinking about how much it hurts.
11. ☐ I keep thinking about how badly I want the pain to stop.
12. ☐ There's nothing I can do to reduce the intensity of the pain.
13. ☐ I wonder whether something serious may happen.

… Total

Appendix 4: Patient Information Flier (Aim 2)

The goal of the research is to determine if providing amino acid supplementation (building blocks for protein) can stimulate recovery and reduce muscle loss after surgery.

Who Can Participate? Patients ages 50 to 80 years old having their first total knee replacement surgery performed by Dr. Jewett, Dr. Lantz, Dr. Mohler, or Dr. Shah may be able to participate.

What's Involved? Each participate will be randomly assigned to either receive the essential amino acid supplement or a placebo, which is a supplement without any essential amino acids. Like a coin flip, you have about a 50-50 chance of receiving the real essential amino acid supplement. You would need to take the supplement two times per day, after breakfast and lunch, one week before surgery and six weeks after surgery. There are also extra tests and procedures you would have as part of this study such as ~~DEXA scans,~~ muscle biopsies~~,~~ and tests that measure your ability to get around before and after surgery.

How long is the study? The study starts about six weeks before surgery and ends approximately six months after your total knee replacement ~~(with an option to participate up to one year after your surgery).~~

Has anyone participated in a study like this before? Yes, Drs. Jewett, Lantz and Shah participated in the pilot data project. The pilot data was published in the Fall of 2013. Dr. Hans Dreyer from the University of Oregon is in charge of the study.

Our preliminary studies suggest taking essential amino acid supplements prior to surgery and for two weeks after surgery prevents muscle loss. Preventing muscle loss is important because improving muscle strength and functional after knee replacement may decrease recovery time; however, this is part of what we are investigating. There is a chance you would receive the placebo (no real supplement), in which case you may not benefit from study participation directly, but you would play an important part in helping us determine whether or not EAA supplementation is beneficial. Study participants will be compensated for their time.

How do I find out more information? Study coordinators will make sure you understand all study-related risks and go over all information with you in detail before your agree to participate.

To find out more information, please ask your doctor and visit our website at www.slocumfoundation.org.

Contact Erin Owen or Tessa Kirkpatrick at the Slocum Foundation 541.868.3232 or 541.868.0658.

SLOCUM Research & Education FOUNDATION

UO IRB Protocol #12272013.024 PeaceHealth Protocol # 551187 May 2017

PARTICIPANT CONSENT FORM

Title:	**Mechanistic approach to preventing atrophy and restoring function in older adults: The long-term follow-up study**

Principal investigators: Hans C. Dreyer, PT, PhD, Brian A. Jewett, MD, Brick A. Lantz, MD, Craig G. Mohler, MD, and Steven N. Shah, MD.

Approved by the following institutional review boards:
Sacred Heart Medical Center at RiverBend.
University of Oregon.

Questions? Please call your study coordinator at 541.868.0658 or 541.868.3217

Why am I being invited to participate in this clinical study?

You are being asked to participate in this clinical research study because you are between 40-80 years of age and scheduled to undergo a total knee replacement (TKR; also known as total knee arthroplasty or TKA) surgery at Sacred Heart Medical Center, RiverBend or Slocum ASC under the care of orthopedic surgeons Brian Jewett, MD, Brick Lantz, MD, Craig Mohler, MD, or Steven Shah, MD.

This study is being sponsored by a grant from the National Institutes of Health (NIH). A total of 80 TKA patients will be recruited for this particular study. This study is one of two clinical studies that seeks to further demonstrate if recovery from surgery for older adults can be improved. The first study was completed in 2016 and included 39 patients who completed all the study activities.

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, please sign the form. Your signature means that you have read this document and have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study and that you are willing to undergo all of the testing outlined below. Participation in this study is voluntary. Please note that this consent form is for the research study only and that consent for surgery will be obtained at a later time by the surgical team.

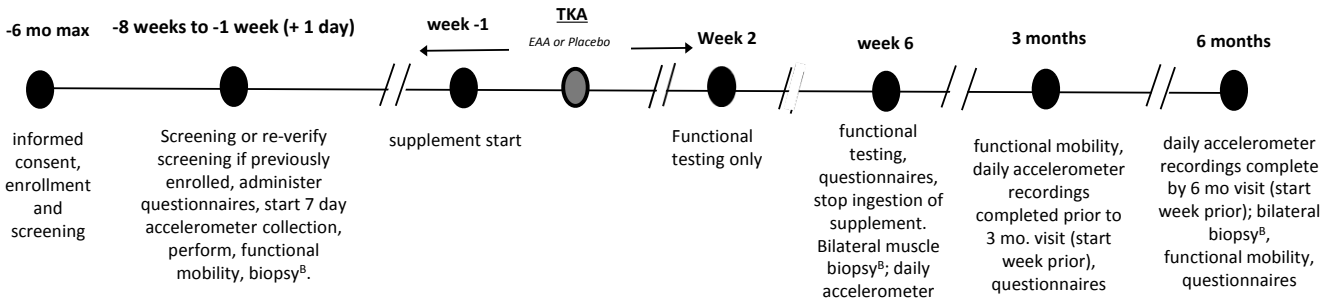
What is the purpose of the study?

This study will determine the effect of ingesting essential amino acids (EAA) twice-daily for 1 week before through 6 weeks after your total knee replacement surgery. Each participant will be randomly assigned to either receive the EAA or a placebo (a non-essential amino acid called alanine) supplement. Like a coin flip, you have about a 50-50 chance of receiving the real essential amino acid supplement. Two groups (EAA or placebo) will then be compared for things such as muscle protein synthesis, muscle architecture and functional mobility. We want to see if supplementing your diet with EAA will reduce muscle loss and boost recovery.

What will happen?

Below is an outline of what we require for your participation in this study and when study activities happen. It is very important that you complete each of the testing sessions, measures, and questionnaires to the best of your ability. To assist you with this a study coordinator will be in regular contact with you during the course of this study. Phone calls by the research staff and regular staff of the Slocum Center for Sports Medicine & Orthopedics will occur often. Below is an overview of the entire study. This provides a general outline of what is involved.

Overview of Aim #2 study timeline, activities, measures and procedures.



Schedule of Events								
Time	**-6 mo max**	**-8 wks to -1 wks (+ 1 day)**	**-1 wk (± 0 day)**	**TKA (Day 0)**	**2 wk (± 1 week)**	**6 wk (± 1 week)**	**3 mo (± 2 weeks)**	**6 mo (± 2 weeks)**
Enrollment	X	X						
Screening	X	X						
Questionnaires		X				X	X	X
Functional Mobility Test		X			X	X	X	X
Accelerometer		XA				X	XA	XA
Supplement*			start →			Stop		
Bilateral Muscle BiopsyB		XB				X		X

Functional testing & biopsy: Performed at the Dreyer Lab, University of Oregon or the Slocum Center.

A Subjects will have the accelerometer ON daily during waking hours (off while sleeping and showers) for a 7 consecutive day period ending at this time point.
BOptional muscle biopsy as per patient choice; Biopsies must be completed a minimum of 4 weeks prior to TKA; patients who are enrolled between -4 weeks and -1 week (+ 1 day) will not be eligible to participate in the biopsy arm.
*Twice daily ingestion of supplement (subject and investigators are blinded to treatment group); Ingestion will occur within1 hour after daily physical therapy.

Study Activities

Study activities as described above and their time points will be scheduled/coordinated with you and your study coordinator. You will be required to make extra trips for study only activities. Your study coordinators will try and minimize these trips by combining as many study activities as possible (within study window) into one visit. They will work with you to determine what works best for your travel needs.

Pre-TKA supplement ingestion (before surgery).

Starting 7 days before your surgery, you will begin supplementation. Each day at approximately 10 AM (but at least two hours after you have eaten breakfast) you will mix two level scoops of supplement into water or beverage of choice (e.g. smoothie) and and drink. You will do this again approximately 2 hours after lunch (~2 PM). If you miss a time point you can make up that time point later in the day however we request that you do so at least 3 to 4 hours apart (supplements in a day) and that you record this in the log book we provide you. Any questions you have please call your study coordinator at the Slocum Foundation. They can be reached at 541-868-0658 or 541-868-3217.

Post-TKA supplement ingestion (after surgery).

Just like before you had surgery you will again ingest supplement twice each day for 6 weeks. This will occur while you are in the hospital (not on day of surgery) and after you are discharged. Each day at approximately 10 AM (but at least two hours after you have eaten breakfast) you will be given your supplement tor drink. This will occur again in the afternoon at approximately 2 PM. If you miss a time point you can make up that time point later in the day however we request that you do so at least 3 to 4 hours apart (supplements in a day) and that you record this in the logbook we provide you. Any questions you have please call your study coordinator at 541-868-0658 or 541-868-3217.

After surgery you will have physical therapy which is routine and prescribed by your doctor. On those occasions when you receive physical therapy treatment you will ingest the supplement within one hour after treatment. If you have treatment by physical therapy in the morning that will be your morning supplement for the day and your next supplement will be at approximately 2 PM. If you have physical therapy in the afternoon then your afternoon (second supplement of the day) will be ingested within one hour of therapy. *This is very important as our research has shown that supplement ingestion is much better for you if taken within one hour of exercise.*

Logbooks.

Over the course of the study we will help you keep track of certain components that are important for us to record. For example the logbook will help you to keep track of when you take your supplement.

The study team will also keep logbooks as part of the study. We will ask you about your demographics (example: age, race/ethnicity), health status, mental health status, medications and any health-related changes or tests that happen to you during the study. We have to track these details to determine how essential amino acid supplementation impacts your recovery after surgery. The study coordinators will help you keep your logbook, and ours, up to date.

Accelerometer.

In order to measure how active you are, we ask that you wear an accelerometer at your waist. Wearing the accelerometer will begin close to when you start the study. The accelerometer will be worn as a piece of clothing and put on in the morning and kept on until you go to bed each day. The accelerometer will be worn at four time points during the study for seven consecutive days:

(1) Once between 8 to four weeks before your surgery
(2) Once at six weeks post surgery
(3) Once at three months post surgery
(4) Once at six months post surgery

This device will help us to gauge the effect of overall physical activity on recovery and how this may explain some of the positive results.

Questionnaires.

You will be asked to complete questionnaires over the course of this study that will help us to understand your health, health-related quality of life, and ability to move around factor into your progress. These questionnaires will be completed before TKA surgery and at 6 weeks, 3 months, and 6 months after surgery. *The questionnaires will help us compare participants in the study over time and help us better understand the role essential amino acid may have on your recovery after surgery.*

Function (ability to move around and strength).

Here are the tests we will ask you to do during this study that will help us measure your ability to get around and how strong you are before and after surgery:

- standardized grip strength,
- standing balance,
- chair stand test,
- timed up-and-go (TUG),
- timed stair ascent (up) and timed stair descent (down),
- four-meter walk and

You will complete each of the above between 8 weeks and 4 weeks before your surgery, and at 2 weeks, 6 weeks, 3 months, and 6 months after TKA surgery.

Grip strength will allow us to assess changes in each hand over time. Standing balance will measure changes over time. The chair stand test measures the time it takes for the participant to stand and sit five times. TUG will measure the time (seconds) needed to stand from a chair, walk 3 meters, turn around, walk back and be seated again. Timed stair ascent (10 steps) and descent will be recorded. We have experience with these functional measures. The 4 meter walk will tell us how long it takes you to walk a short distance (4 meters). Functional tests will be performed at the University of Oregon or the Slocum Center.

Completing the functional mobility assessment is the main component of this study that we are looking to change in a positive sense with supplementation. Older adults who have this surgery may be able to do much more if the supplementation helps regain mobility.

About the biopsies (optional).

This study involves optional biopsies. These biopsies will only be performed if you elect to have them done and if the biopsy can be scheduled for at least 4 weeks before your total knee replacement surgery. If a biopsy cannot be scheduled at least 4 weeks prior to your surgery, you may still be able to participate in this study, but you would not have the choice whether to participate in the biopsies or not. If it is too close to your surgery date, you are eligible to participate in all the study activities, and take the supplement, but would not have any biopsies before or after surgery.The following information is if you elect to have the biopsies performed.

A small incision about the size of this dash "___" approximately at mid-thigh, through which a needle about the size of this letter "**O**" will be advanced into the muscle. A piece of tissue will then be removed. This will be done on each leg.

Your biopsies will be performed by the Principal Investigator, Dr. Dreyer, who is running this trial. Dr. Dreyer has performed over 500 biopsies. The biopsies will be performed by Dr. Dreyer in his lab at the Center for Medical Education and Research, or at the Slocum Center, in the morning before you've had food (fasted state). This procedure involves the taking of a small piece of muscle tissue from your leg. The skin is cleaned and made sterile, and the skin and tissue below are injected with local anesthetic, lidocaine (numbing medicine), to eliminate pain. Afterwards, the skin will be closed with a single stitch and/or steri-strips and a dressing. The stitch, if used, will be removed by research study staff in about 7 days (plus or minus one day to accommodate your schedule).

If you choose, you will have biopsies at three time points, one biopsy in each leg (total of six):

(1) 4 to 8 weeks before surgery
(2) 6 weeks after surgery
(3) 6 months after surgery

The Dreyer Lab will be measuring your muscle weight, comparing the wet biopsies to dried muscle samples. The samples will tell the study team how much edema (fluid accumulation) you have in your leg before and after surgery.

From your muscle biopsies we will isolate primary muscle cells called satellite cells. Under the right conditions in cell culture, these primary cells, isolated from you muscle tissue samples, can increase in number and form primitive muscle cells. Recently, this type of work has shown that these cells can inform us about how your muscles may respond to various conditions. We will perform primary cell isolation in order to conduct experiments on your muscle cells that we could not perform on you (For example, we can block metabolic processes from happening and evaluate the impact on muscle tissue) and increase the amount of muscle tissue we have to work with. You will be fasting (no breakfast) before your muscle biopsies.

This research will help us to learn much more about how your muscle cells respond to treatment over the course of the clinical trial.

The Primary Cell Isolation Experiment is Not Long-term Storage of Your Cells. The primary cell isolation technique is not a way to develop perpetual (long-term) cell lines. Once we isolate primary cells we will keep them frozen at -80°C until we are ready to perform cell culture experiments on them. However, once they are in cell culture, they have a finite life span and will expire within 2 to 4 weeks. After the experiments on your cells are completed they will be dead already or destroyed.

Muscle biopsy option – For patients who are able to have biopsies at least 4 weeks prior to surgery only

You have the option to choose whether you would like to participate in this research study with or without having the muscle biopsies. You do not have the option to have biopsies if your date of surgery is less than four weeks away and/or you cannot schedule your biopsy to be completed at least four week prior to surgery.

You may choose to participate in the study having muscle biopsies at three different time periods or you may choose to forego the muscle biopsies and continue participation in the rest of the study without them.

If you decide to have muscle biopsies, you still have the right to change your mind at any point in the study, whether you have already had a biopsy or not and still continue participation.

You choose: (check box and initial)

☐ to have muscle biopsies _____

☐ to **not** have muscle biopsies _____

How long will I be in the study?

You will officially be in the study from the date you sign this consent form, which could be up to six months before surgery until the last study activity is completed six months after surgery. Your study-related activities don't begin until approximately 8 weeks before surgery and end approximately 6 months after your surgery. Most people will be actively participating in the study for about eight months.

IMPORTANT: Participation in this study will not affect your hospital length of stay, which is determined by your orthopedic surgeon.

What are the risks of participating in this research?

This study may include risks that are unknown at this time. Possible risks and discomforts you could experience during this study include:

Risk from biopsies. The potential risks of this procedure are some pain, bleeding, bruising, infection, and scar at the site of biopsy. Careful sterile technique should reduce the likelihood of any of these complications. The risk of bleeding from the biopsy site is 0.2%; the risk of bruising or blue-and-black mark is 1.4%; and the risk of infection is so small that the precise number is unknown. Additionally, you may experience numbness around the area of the biopsy site (approximately 2x2 inches in area), which will likely go away with time (sensation returns) but in very rare instances sensation may never return. The risk that you experience numbness is less than 0.5% and the risk that the numbness never goes away is much less. Pre-TKA (4-8 weeks prior to surgery) and Post-TKA biopsies (6 weeks and 6 months) will be performed using local anesthetic (lidocaine), which will remove or eliminate sensation of the biopsy. After a biopsy you have a 50% chance of experiencing soreness at the site of biopsy for 24 to 48 hours. Over the counter medication will be enough to eliminate any potential pain from the biopsy site. The scar will be approximately long like this dash "___". *We have performed muscle biopsies on over 100 older adults during and after TKA without side-effects.*

It is important to note the biopsies occur while you are fasting (no food since dinner the night before). On the morning of your post-operative biopsies you may have to delay taking your pain medication, or risk upset stomach if you take your pain medication without food. The study team can accommodate post-operative biopsies as early as 6:00am to help minimize any potential discomfort.

Risk from falls. There is a possibility that you may fall and hurt yourself during this study. This is mentioned because your knee pain affects your ability to control your muscles. Also, it takes time to heal from this type of surgery and we are asking you to perform physical tasks that may be challenging to you. You will be supervised at all times when completing the tests that help us determine your ability to move and your strength.

Pregnancy. The risks of the amino acid supplement to the human embryo or fetus have not been studied. Women who are pregnant are not eligible to participate in this study. Women of potential childbearing age will be asked if they are or could possibly be pregnant. It is possible a hormone pregnancy test will be administered to verify pregnancy status. The study staff can answer any questions you may have about this process.

Are there benefits to taking part in this study?

You will likely receive no direct benefit from your participation in this study. Essential amino acids have been shown to reduce muscle loss in persons having TKA such as yourself. For example, in a study we recently completed we found that patients in the EAA group lost less muscle from their legs as compared to those in the placebo group. This may be considered a potential benefit. However, that study included only 28 participants total, which clinically is considered a very small sample. We repeated this study with 39 participants in 2014 – 2016 and again found the group taking EAA lost less muscle than the placebo group. Thus, the purpose of this study is to replicate those findings in a much larger group of participants, and determine how EAA supplementation may be working at the cellular level. Neither you nor the investigators working directly with you will be aware of which study group you are in (EAA or Placebo). This is called a placebo controlled double blind study for this reason.

Will you receive new information about the study?

You will be told about any new findings/information that might change your decision to be in this study. You may be asked to sign a new consent form if this occurs to ensure you full understand the changes.

What other choices do I have if I don't participate in this research study?

This research is being conducted to gather information. You are free to choose not to take part in this study. Should you decide to withdrawal participation, your surgeon and care providers will care for you according to their routine standard care procedures. Payment for your routine care after withdrawal from the study will be the same as if you did not participate in the study, which you will have to work out between your surgeon, care facilities and your insurance company.

Are there any costs associated with this research study?

You will not pay for any tests or procedures that are performed for the purposes of this research study. You will be compensated for participating in the study. This money is to compensate you for your time and any inconvenience it presents to you. If you do not complete the study, you will receive a reduced amount of money based on study timeline events completed:

Compensation plan if you choose to have muscle biopsies (maximum $300):

- $100.00 for completion of preoperative biopsy
- $100.00 for completion of six week postop biopsy
- $100.00 for completion of six month postop biopsy (study completion)

Please note that you will not be compensated for the two week and three month study activity time points. Compensation only reflects the study time points in which you have biopsies.

Compensation plan if you choose NOT to have muscle biopsies (maximum $100):

- $20.00 for completion of preoperative testing
- $20.00 for completion of two week postop testing
- $20.00 for completion of six week postop testing
- $20.00 for completion of three month postop testing
- $20.00 for completion of six month postop testing (study completion)

If you decide to withdrawal participation from the study or your physician or research staff withdrawals your participation, you will be compensated for the maximum time-point above completed. For example, if you complete your six week post-op biopsy, you would be compensated $200.00. If you complete your six week post-op testing, you would be compensated $60.00.

Please note, compensation from participation in Human Subjects Research studies is taxable income. If your compensation totals $600 or more in a calendar year, the University of Oregon is required to report the income to the IRS. The University requires its departments to tack participant compensation and may contact you to complete a Form W-9 for tax reporting purposes. Because of the federal and University tracking requirements, your name will be associated with participation in research. Department and University administrators will have access to this information, but will not have access to research data.

Where does the study supplement come from?

The study team has learned from the first two EAA studies we have done that essential amino acids have a very bitter taste. To overcome this barrier, we have partnered with a company called MEND Nutrition, Inc. (“MEND”) who adds all natural flavoring to the supplement so patients are able to take it without too much trouble. MEND worked directly with our team to create a custom supplement that meets our requirements regarding the composition of EAAs we are studying. MEND has provided the supplement to our study for only the cost of shipping. In exchange for making our supplement and the placebo for us to study, the Slocum Center will sell the other MEND products that are commercially available nutritional supplements. The profits from the sale of the other MEND nutritional products will go back to the nonprofit Slocum Foundation to support our research program. Neither the Slocum Center nor the physicians affiliated with this study profit from the sale of MEND nutritional products at Slocum.

Who can answer my questions?

You may talk to your orthopedic surgeon, Dr. Brian Jewett, MD, Dr. Brick Lantz, MD, Dr. Craig Mohler, MD, or Dr. Steven Shah, MD (all principal investigators), or to the co-principal investigator Dr. Hans Dreyer, PT, PhD. You will have regular contact with our study coordinators, who will call you on a regular basis over the course of the study. Any questions you have please call your study coordinator at 541-868-3232.

What are my rights if I take part in this study?

Taking part in this research study is your decision. You do not have to take part in this study, but if you do, you can stop at any time. Your decision whether or not to participate will not affect your relationship with your physician, or the Sacred Heart Medical Center/PeaceHealth or your surgery in any way.

You do not waive any liability rights for personal injury by signing this form.

All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. In spite of precautions, you might develop medical complications from participating in this study. If such complications arise, the researchers will assist you in obtaining appropriate medical treatment but PeaceHealth, Slocum and University of Oregon do not provide financial assistance for medical or other costs.

If you are physically injured because of the project, you and your insurance company will have to pay your doctor bills. If you have no insurance the cost of treatment will be yours.

Your relationship with University of Oregon, PeaceHealth, and the Slocum Center for Orthopedics & Sports Medicine will not be impacted by your participation in this study.

If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel
Office of the President
1226 University of Oregon
Eugene, OR 97403
(541) 346-3082

Research Compliance Services
5237 University of Oregon
Eugene, OR 97403
(541) 346-2510

A law called the Oregon Tort Claims Act limits the amount you can receive from the State of Oregon if you are harmed.

What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Participant identities will be kept confidential by assigning you a “participant identification number”. The names associated with each participant identification number will be kept in a locked file cabinet in Dr. Dreyer’s office area.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

UO Research Compliance Services, PeaceHealth Institutional Review Board, and/or authorized representatives of the National Institute of Health (NIH) may need to review records of individual participants. As a result, they may see your name, but they are bound by rules of confidentiality not to reveal your identity to others. The Food and Drug Administration (FDA) may inspect records associated with this study.

Should you choose to have muscle biopsies, all of your muscle tissue will be used for study analysis. For example for muscle we will look at changes in mRNA, cell signaling and changes in cell structure. The researchers may store the information gathered during this study indefinitely.

Information about your participation in the research study, including your willingness to consent to study participation will be included in your Slocum medical record. When your study coordinators are tracking your participation, they may note any important health events and correspondence with you in your electronic medical record at Slocum. Your surgeon needs the information documented to manage your care and monitor your health and well-being.

The supplement is being supplied by MEND Nutrition, Inc. ("MEND"). MEND is providing the supplement for minimal cost and is interested in the results of the study. MEND will be given de-identified study information for functional testing and patient questionnaires related to your study outcomes only. None of the information provided to MEND will contain information that could identify you. The information will be similar to what is published as study results in academic or medical journals.

If I agree to participate, who can decide to end my participation?

You may decide to end your participation at any time. The investigators may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

Your well-being is our primary concern. Throughout the study, we will be monitoring you very closely. Your surgeon, or the study team, can end your study participation at any time if it is in your best interest to protect your health and well-being.

Having TKA surgery is a requirement of participation in this study. If for any reason you will not continue with the planned surgery or if the surgery is delayed, you may be withdrawn from continued participation or your participation disrupted until the surgery is rescheduled. In addition, should an individual become pregnant while enrolled in the study, participation would be discontinued as it is not known whether the investigational drug (i.e., EAA supplement) is safe for use by pregnant women.

Consent to Participate in the Research Study

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this study. If you have questions regarding your rights as a research participant, contact University of Oregon Research Compliance Services, 5237 University of Oregon, Eugene, OR 97403, (541) 346-2510 or the PeaceHealth Institutional Review Board, (541) 686-6949.

You will be asked to sign a separate form that allows the study team members to communicate about you and your health information during the study.

Your signature indicates that you have:

- read and understand the information provided above,
- all of your questions have been answered,
- you willingly agree to participate,
- you may withdraw your consent at any time and discontinue participation without penalty,
- you understand that should you withdraw your consent at any time you will continue to receive routine standard of care as determined by your surgeon and care providers,
- payment for the care you receive is the same as if you did not participate in the study,
- you will receive a copy of this form, and
- you are not waiving any legal claims, rights or remedies

_____ _____
(Date and Time) (Signature of Participant)

_____
Printed Name of Participant

_____ _____
(Date and Time) (Signature of Individual Obtaining Consent)

_____
Printed Name of Individual Obtaining Consent