PROTOCOL

Title: Effect of a ghrelin receptor agonist on muscle and bone

HNRCA # 3035

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List amendments

Amendment 1: Change GE Lunar to Hologic DXA scanner; expand reasons for discontinuing a subject in screening and intra-study ICFs; change vendor of D3-creatine; modify pill dispensing plan; correct several typos and inconsistencies

Amendment 2: Add two new recruitment letters for TMC Renal and General.

Amendment 3: Screening 1 medical history and physical exam may be moved to Screening visit 2 on occasion to facilitate scheduling; increase number of subjects to 250; reformatted compliance calendars.

Amendment 4: Post-COVID safety changes; single screening visit; redefine sarcopenia; modify recruitment.

Amendment 5: Modification of evening meal prior to urine collection for D_3-creatine measurement.

Amendment 6: Add new recruitment letter for TMC patients identified by the Tufts CTSI.

Amendment 7: Add FFQ and waist circumference at month 12.

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Protocol summary

Synopsis
Adults with both osteopenia and sarcopenia (osteosarcopenia, OS) have greater risk of falls and fractures than those with osteopenia or sarcopenia alone. Drugs are available to reduce fracture risk but currently exercise is the only effective strategy to combat muscle loss. Unfortunately, the majority of adults who start a self-monitored exercise program drop out after 6 months and other options are needed. Ghrelin receptor agonists have been under development to treat anorexia and weight loss in patients with cancer cachexia. The agonist anamorelin has significantly increased weight and lean tissue mass in these patients. Anamorelin mimics the hormone ghrelin which not only increases appetite, but also acts on the pituitary to increase pulsatile growth hormone (GH) secretion. Pulsatile GH stimulates the production of insulin-like growth factor 1 (IGF-1) which is anabolic to both muscle and bone. GH levels decline with age and this is thought to contribute to the age-related muscle and bone losses in adults. Our central hypothesis is that anamorelin will increase muscle mass, improve muscle function, and increase bone formation in adults with osteosarcopenia. To test this hypothesis, we will conduct a randomized, double-blind, 2-armed, parallel-group intervention trial in 32 osteosarcopenic men and postmenopausal women age 50 and older. Participants will be randomized to anamorelin (100 mg per day) or placebo and treated for 12 months. The primary endpoint is change from baseline in muscle mass by D_3-creatine dilution. Secondary endpoints are changes from baseline in: appendicular lean tissue mass/ht^2 (ALM/ht^2) measured by dual-energy x-ray absorptiometry (DXA); the bone formation biomarker, amino-terminal propeptide (P1NP), total body lean mass by DXA. Exploratory outcomes are changes in isokinetic leg strength, grip strength, and muscle performance (Health ABC-Physical Performance Battery (HABC-PPB), serum IGF-1 and C-telopeptide (CTX), and spine and hip bone mineral density (BMD). The proposed treatment supplies the anabolic stimulus to build both muscle and bone. Anamorelin has not been tested in adults with osteosarcopenia. We plan to evaluate anamorelin in osteosarcopenic adults because they are most in need of treatment and most likely to benefit. Data obtained from this study are critical to determine the feasibility and guide the design of a definitive trial to evaluate this ghrelin receptor agonist as potential therapy to mitigate the dual hazards of osteopenia and sarcopenia.

Schedule of activities
After a single visit screening process, eligible subjects will be enrolled in this study for 12 months each. They will visit the HNRCA as follows: 1) enrollment visit - sign consent form, receive oral D_3-creatine dose, and have practice leg endurance measurement; 2) baseline visit – medical history and physical measures, DXA scans, blood draw, pill dispensing and receive first drug dose, and have pre-dose,1-hr post first dose, and 4-hr post first dose ECGs (ECGs this visit only); 3) 2 mo visit – repeat baseline measures (except D_3-creatine), pill count; 4) 6 mo visit – repeat baseline measures (except D_3-creatine), pill count; 5) 3 days prior to final 12 mo visit – receive oral D_3-creatine dose; 6) 12 mo visit – repeat baseline measures and pill count. Additionally, subjects will be called at 4, 8, and 10 mo to reinforce pill compliance and to answer any questions they may have.

INTRODUCTION

Rationale This study is an initial evaluation of the effectiveness of the novel ghrelin receptor agonist, anamorelin, on increasing muscle mass, strength, and function and enhancing bone formation in men and postmenopausal women with low bone and muscle mass (osteosarcopenia, OS). Anamorelin has been tested in normal adults and in patients with cancer cachexia but it has not been tested in patients with osteopenia, sarcopenia, or OS.

Background Osteopenia and sarcopenia result from the age-related losses of bone and muscle mass that occur at about 1-2% per year after age 50 years (1-3). Muscle strength declines more rapidly than muscle mass (4-6), nonetheless the loss of muscle mass is a major factor in the decline in strength that occurs with aging (7-10). The definition of osteopenia is standardized, and refers to a BMD T-score of -1 to -2.5. Currently there is no standardized definition of sarcopenia. Mounting recent evidence indicates that lower levels of functional measures, grip strength and gait speed, are significant predictors of falls and hospitalizations,

whereas ALM measured by DXA is not (11, 12). Accordingly, the Sarcopenia Definitions and Outcomes Consortium (SDOC) recommends that sarcopenia definitions include grip and gait speed but not ALM by DXA (11, 12). The SDOC indicates that other measures of muscle mass including D_3-creatine are promising based on recent evidence that decreases in muscle mass measured by D_3-creatine dilution are associated with significant declines in functional status including grip strength and gait speed (13). The prevalence of sarcopenia increases after age 50 years to reach 50% among seniors age 80 and older (14). Biochemical markers of bone resorption increase with age and the increase results in bone loss (15, 16).

The pathophysiologies of osteopenia and sarcopenia have overlapping features related to the mechanostat theory (17) and to biochemistry (GH, IGF-1, IL-6, and osteocalcin) (18, 19). It is therefore not surprising that bone and muscle losses in adults often track together. A study in males age 60 yrs+ showed a 9-fold greater risk of low BMD in individuals diagnosed as sarcopenic and an 8-fold risk in those diagnosed as pre-sarcopenic (20). Drey et al. found that adults with OS, in contrast to those with a single component (osteopenia or sarcopenia), had reduced muscle strength when compared with controls (21). Sarcopenia has been widely associated with functional impairment (22), falls (13, 23), loss of independence (24) and mortality (25).

Anamorelin was initially developed as a treatment for cancer cachexia. Two Phase 3 trials involving a total of 979 patients with advanced lung cancer and associated cachexia have demonstrated substantial gains in body weight and lean tissue mass over 3 months of treatment with anamorelin (26). The anamorelin groups gained 0.6 and 0.8 kg of ALM, relative to placebo and they gained 1.7 kg and 1.8 kg, respectively, in total lean mass (26). Within the anamorelin groups, ALM gains accounted for the vast majority of lean mass gains (85% and 95%) and 39.5% and 65.3% of total weight gains in the two trials (26). These gains would offset the average loss in lean mass occurring over a 3-year period in adults. Anamorelin may also increase total body lean mass as assessed by the D_3-creatinine dilution method (27), but this has not been tested. There was a trend toward increased grip strength in these trials (26), particularly among patients age 65 years and older. In a meta-analysis of ghrelin agonist trials, a significant increase in grip strength was observed in the 5 studies that included this measurement (28). It is plausible that gains in lean tissue mass and strength on anamorelin would be greater with a treatment duration >3 mo. This possibility is supported by the recent report of the Phase 3 Safety Extension study in which 513 cancer patients continued their treatment for an additional 3 mo. During the extension, weight gain continued at the same rate that it had in the first 3 mo of treatment (29). ALM was not assessed in the extension study. In the Phase 3 trials, anamorelin had a very good safety profile. Grade 3-4 hyperglycemia occurred in <1% of patients taking anamorelin (26). Low-grade (Grade 1-2) gastrointestinal disorders, mainly nausea, occurred in 6% of patients taking anamorelin in one trial and in 2% in the other (26). In the extension study involving 6 months of treatment, anamorelin continued to be well tolerated (29).

Anamorelin has potential favorable effects on bone. Pulsatile GH secretion declines with aging and this likely contributes to bone loss and fracture risk. GH stimulation could counter bone loss as it stimulates osteoblast proliferation and differentiation *in vitro* (30). The effect of anamorelin on bone turnover or other bone parameters has not been reported, but treatment with another ghrelin receptor agonist, MK-669, for 9 weeks significantly increased the bone formation markers, serum osteocalcin by 29% and bone-specific alkaline phosphatase by 10.4%, and it also increased urinary N-telopeptide by 22.6% in functionally impaired older adults (30).

Anamorelin may be more effective on muscle than exogenous GH or IGF-1. Anamorelin stimulates the release of GH in its normal pulsatile pattern. Pulsatile GH affects muscle through increases in circulating and intramuscular IGF-1 (31). Circulating IGF-1, which is largely of liver origin, stimulates muscle protein synthesis by binding to IGF-1 receptors on myocytes and activating the mTOR pathway (31). In addition to increasing circulating levels of IGF-1, GH binds to its receptors on myocytes to stimulate the production of intramuscular

IGF-1 which acts locally to inhibit myostatin and promote satellite cell (myocyte) activity (32). Exogenous nonpulsatile GH treatment increases lean mass (33) but its use is limited by side effects (edema, arthralgias, paresthesias) that have not been seen in the anamorelin trials. Exogenous recombinant human (rh) IGF-1, unlike anamorelin, has no impact on intramuscular IGF-1 production.

Objectives and endpoints

Our central hypothesis is that anamorelin will increase muscle mass. We will also assess the secondary and exploratory outcomes described below in OS adults. To test this hypothesis, we will conduct a double-blind, 2-armed, parallel-group intervention trial in men and postmenopausal women age 50 and older with OS. Participants will be randomly assigned in a one to one ratio to: anamorelin or placebo and treated for 12 mo.

Specific aims

1. Primary aim To determine and compare the effects of anamorelin vs placebo on muscle mass by D_3-creatine dilution in OS adults. *Hypothesis: Anamorelin will increase muscle mass.*

2. Secondary aims

2A. To determine and compare the effects of anamorelin vs placebo on serum procollagen 1 intact N-terminal (P1NP), a biochemical marker of bone formation. *Hypothesis: Anamorelin will increase P1NP.*

2B. Determine whether changes in muscle mass and P1NP are predicted by sex, age, Health ABC-Physical Performance Battery (HACB-PPB) score, protein intake, or adherence to the intervention.

2C. Describe the safety and tolerability of anamorelin.

2D. Determine and compare the effects of anamorelin vs placebo on appendicular and total body lean mass assessed by DXA. *Hypothesis: Anamorelin will increase appendicular and total body lean mass.*

3. Exploratory aims

3A. To gain additional information on the effects of anamorelin, we will describe and compare the effects of anamorelin on muscle strength (isokinetic leg strength and handgrip) and muscle performance (HABC-PPB). *Hypothesis: Anamorelin will increase strength and performance.*

3B. To examine group changes in serum IGF-1, in the bone resorption marker, serum C-telopeptide (CTX), and in spine and hip bone mineral density (BMD). *Hypotheses: Anamorelin will increase serum IGF-1, serum CTX (as a result of coupling to the increase in P1NP), and BMD of the spine and hip.*

Data obtained from this pilot study are critical to determine the feasibility and guide the design of larger studies to evaluate ghrelin receptor agonists as potential therapies to mitigate the dual hazard of OS.

Endpoints:

Primary: muscle mass by D_3-creatine dilution

Secondary: P1NP, appendicular and total body lean mass by DXA

Exploratory: isokinetic leg strength, handgrip, HABC-PPB, IGF-1, CTX, spine and hip BMD

RESEARCH DESIGN

A. Study design

This is a double blinded, placebo controlled 2-armed parallel group trial testing the effect of anamorelin versus placebo on 12-month changes in muscle mass (primary) and the above described secondary and exploratory endpoints in community-dwelling older adults with OS. Up to 250 will sign the screening consent form in order to enroll 32 subjects and to have 24 completers. Subjects will be normal weight and overweight men and postmenopausal women age 50 years and older with OS.

B. Schedule of activities

Visit schedule	Enroll Day -3	0	2 mo	6 mo	Day 361	12 mo
Consent	X					
Medical history		X	X	X		X
Weight		X	X	X		X
Blood pressure		X		X		X
ECGs before and 1 & 4 hr post dose		X				
Diet assessment[a]		X				X
Questionnaires[b]		X		X		X
Muscle strength[c]	X	X	X	X		X
Muscle perform.[d]		X	X	X		X
DXA scans[e]			X	X		X
Study blood[f]		X	X	X		X
Dispense pills		X	X	X		
Safety measures[g]		X	X	X		X
Adherence[h]			X	X		X
D_3-creatine dosing[i]	X				X	
Fasting spot urine[j]		X				X
Waist circumference						X

Footnotes

[a] By food frequency questionnaire
[b] Physical activity and SarQoL®
[c] Isokinetic leg endurance on each visit and hand grip on 0, 2, 6, and 12 mo
[d] HACB-PPB at 0, 2, 6 &12 mo
[e] DXA total body scans at 2, 6, &12 mo; spine and non-dominant hip scans at 12 mo (screening scans will serve as baseline)
[f] Serum P1NP at 0, 2, 6, and 12 mo;
serum IGF-1 and CTX at 0 and 12 mo; serum 25(OH)D at 0 and mo 6; and alpha-acid glycoprotein at visit 0;
archive 4 aliquots serum and 1 of plasma at 0 and 12 mo.
[g] Safety monitoring: fasting plasma glucose & serum AST/ALT at 0, 2, 6, and 12 mo; symptoms and other AEs documented
[h] By pill diary and counting returned pills
[i] Administer 60-mg oral dose of D_3-creatine
[j] Collect fasting spot urine for D_3-creatine dilution determination

In addition to the above study visits, we will contact participants by telephone at 4, 8, and 10 months to determine whether they have any study related concerns and to remind them of the importance of taking one study pill per day. The content of these calls will be documented on the Call/comment Log.

C. Inclusion and exclusion criteria

Inclusion criteria: Ability to sign informed consent form; community dwelling men (who are sterile or agree to use contraception throughout the study) and postmenopausal women (no menses for 5 years; early postmenopausal women are ineligible because their bone turnover rate is changing rapidly); age 50 years and older; sarcopenia defined as maximum grip strength <35.5 kg (men) and <20 kg (women) in either hand (excluding hands with severe pain or recent surgery) and/or gait speed <0.8 m/sec (11); osteopenia defined as spine (at L1, L2, L3, or L4) or non-dominant total hip or femoral neck BMD T-score between -1.0 and -2.5; mini-mental state examination (MMSE) score >21.

Exclusion criteria – *Conditions:* BMI > 30 kg/m^2 (obese are ineligible because anamorelin may cause weight gain); osteoporosis of the spine or hip by DXA scan (specifically, T-score ≤ -2.5 at two lumbar vertebrae or at the total hip or femoral neck, as recommended by the International Society for Clinical Densitometry [ISCD]); current participation in a fitness program or weight loss program; advanced knee osteoarthritis (OA) or other conditions preventing strength or function testing; lower extremity fracture in the last year; diabetics taking insulin or sulfonylureas and subjects with a fasting blood sugar on screening >150 mg/dl; inadequate hepatic function defined as AST and ALT levels > 2 x upper limit of normal at screening (>74 and >68 MU/ml, respectively); untreated thyroid or parathyroid disease; significant immune disorder; eGFR<30 ml/min; any clinically meaningful ECG abnormality on screening or baseline; Crohn's disease; active malignancy or cancer therapy in the last 3 years (except nonmelanoma skin cancer); non-English speaking subjects (we can't be confident that non-English speaking subjects could accurately complete the diet assessments which are critical to the integrity of the study); allergy to components of the study interventions; other condition or abnormality in screening labs at discretion of the study physician (the PI); *Medications:* osteoporosis treatment – teriparatide, abaloparatide, raloxifene, denosumab, or romosozumab in the last 12 mo or a bisphosphonate in the last 2 years; Tamoxifen in the last 6 mo; since anamorelin is mainly metabolized by CYP3A4, candidates will be excluded if they are taking strong CYP3A4 inhibitors within the previous two weeks (ketoconazole, clarithromycin, itraconazole, nefazodone, telithromycin); use of drugs that may prolong the PR or QRS interval durations, such as any of the Class I/Sodium (Na+) Channel blocking antiarrhythmic medications (e.g. flecainide, procainamide, propafenone, quinidine); drugs with high affinity to AAG and therefore with potential to displace anamorelin from binding (e.g., carvedilol, chlorpromazine); inhibitors of P-glycoprotein (e.g., verapamil, quinidine), and inhibitors of OATP1B3 (e.g., cyclosporine, rifampicin); CYP3A4 inducers (e.g., rifampin); oral or IV glucocorticoids (>10 days in the last 3 mo); gonadal hormones (vaginal estrogen okay); drugs to promote weight loss or gain; or TNF-α inhibitors (e.g., adalimumab, adalimumab-atto, certolizumab pegol, etanercept, etanercept-szzs, golimumab, infliximab).

D. Recruitment, prescreening, and screening

Recruitment methods

We will identify appropriate potential candidates (i.e., those who meet our age criteria and whose participation in prior studies would not make them ineligible) in the HNRCA Recruitment database, and send them a recruitment letter. We will send a modified recruitment letter to potential candidates who received a recruitment letter for this study >1 year ago, to reassess their interest. We have performed grip strength, gait speed, and spine and hip DXA assessments on hundreds of older adults who are in this database and we will initially target recruitment to those who have indicated that they wish to hear about new studies and who meet the BMD and grip strength and gait speed criteria for this study. We will also send recruitment letters to individuals whose electronic medical record at Tufts Medical Center (TMC) indicates that they are likely to have sarcopenia or osteopenia. We will query the Tufts Medical Center's Research Data Warehouse (TRDW) (https://www.tuftsctsi.org/research-services/informatics/tufts-medical-center-research-data-warehouse/) to identify eligible patient population. The TRDW is a centralized clinical data warehouse that aggregates clinical information from various Tufts Medical Center's systems. We will query the system using the ICD10 (CPT, [other]) codes for concepts required to conduct a study. If feasibility is confirmed, we will request patient names, contact information, demographics, and diagnostic codes to facilitate recruitment. Once potential participants are identified we will use IRB approved recruitment materials for the process.

One letter will be tailored to endocrine patients and sent by a member of the Endocrine Division and another generic letter that is applicable to all other medical specialties will be sent by Drs. Dawson-Hughes. Interested individuals may call for more information about the study and their potential eligibility (opt in). One follow up call will be made to those people who do not reply. A single voicemail will be left for those who do not answer the telephone.

We will also recruit subjects with use of mailing lists purchased from Act One Lists, Marblehead, MA (available by age, sex, and zip code). This involves contacting men and women in the Boston area by mail with a brief description of the study and the inclusion/exclusion criteria. Interested candidates will call. An advertisement will be run in local newspapers and in the Tufts Medical Center Newsletter, giving a call-in number to the Bone Metabolism Lab for more information, as above. A brief study description will be posted on the HNRCA website. Flyers with a brief study description and contact number will be posted throughout Boston and neighboring cities in supermarkets, churches, subways, buses, and senior centers. Written permission will be obtained from supermarkets, churches, senior centers, and Tufts Medical Center before flyers will be placed there. These permissions will be collected and retained by the Bone Metabolism Laboratory. For subway and bus ads, we will purchase the advertising space/time and submit our IRB-approved ads to the MBTA. Posting of IRB-approved, study-specific flyers on bulletin boards at HNRCA is the responsibility of the Recruitment Department, and their posting of such flyers constitutes HNRCA permission.

Telephone Prescreening

Interested candidates will be invited to call our dedicated line at the HNRCA. They will have a prescreening telephone interview about their medical history, height and weight. Interested and potentially eligible candidates will be invited to HNRCA for screening.

Screening

Screening and enrollment procedures as well as documents related to MRU standard policies and procedures, approved under IRB #6701, will be used for this protocol.

Screening visit – The standard health screen will be administered remotely to reduce the length of the screening visit. Upon arrival for the screening visit, candidates will sign the consent form and have grip strength and gait speed measurements. If they qualify as sarcopenic [grip <35.5 kg (men) and <20 kg (women) and/or gait speed < 0.8m/s], they will have spine, non-dominant hip, and total body DXA scans. If they qualify as osteopenic by the WHO criteria (T-score between -1 and -2.5 at 1 or more lumbar vertebrae or at the total hip or femoral neck), they will have a MMSE and physical measures (blood pressure, temperature, heart rate, and weight) followed by a fasting blood draw for chemistry screen and CBC and an ECG. If, during the screening process, a candidate is determined to be ineligible for the study, further screening assessments will be discontinued.

Participants who pass the full screening evaluation will be enrolled.

E. Anamorelin and placebo interventions

Participants will take one pill per day in the morning, at least one hour before breakfast to ensure optimal absorption. The anamorelin dose of 100 mg was selected because it is the dose that was effective and safe in the cancer cachexia trials cited above. The IND # for anamorelin use in this trial is 1327341. The placebo tablets will contain microcrystalline cellulose. Instructions on when to take the pill will be included on their compliance calendars.

The 100 mg anamorelin tablets and identical appearing placebo tablets will be provided by Helsinn Therapeutics, Inc (Lugano, Switzerland). The anamorelin and matching placebos will be shipped in bulk to the

Tufts Medical Center South Basement Pharmacy (attn.: IDS). The drug will be stored at room temperature. The storage life of anamorelin is 5 years. Pharmacy will record the date the drug is unsealed and the expiration date. The date it is opened will be recorded. The Pharmacy will use one NCI Accountability Log for overall shipment and another for subject level accountability.

Pharmacy requires written orders before dispensing. The orders will contain the Protocol name, IRB #, PI name, subject name, DOB, Allergies, Subject ID, visit #; in addition to the prescription and directions and number of tablets to be dispensed (70 pills at baseline, 130 pills at 2 mo, and 190 pills at 6 mo.; note that each dispensing will contain 7-9 extra pills to cover any needs for rescheduling). Each dispensing of capsules will contain an outer label and an inner label. The outer label will be retained by Pharmacy.

Outer label content: Rx # assigned by Pharmacy; PI's Name; Subject name and ID; Date dispensed; Expiration date; either ANAMORELIN 100 MG or PLACEBO; quantity (either 70, 130, or 190).

Inner label content: Rx # assigned by Pharmacy; PI's Name; Subject name and ID; Directions: take one pill by mouth each morning at least 1 hour before breakfast; Date dispensed; Expiration date; "I ANAMORELIN 100 MG/PLACEBO tablet"; quantity (either 70, 130, or 190); and sticker indicating "For investigational use only".

Drug disposition at end of study: The TMC Pharmacy will destroy any unused pills. They will notify Helsinn Therapeutics when this has been done.

F. Measurement procedures

Baseline and follow-up medical history

On the baseline (month 0) visit, subjects will be asked questions about their medical history and demographics including race, ethnicity, education level, and marital status (see baseline medical history and Demographics forms). The follow-up medical histories taken on each subsequent visit focus on recent medical history and medication use (see follow-up medical history form).

Performance and strength measurements

HABC-PPB - The short physical performance battery (SPPB) captures domains of lower extremity strength, endurance, and balance; it is highly predictive of subsequent disability (34)**.** To broaden the applicability, the Health ABC investigators expanded the SPPB by increasing the duration of balance stands (to 30 seconds), adding a single foot stand, and adding a 6-meter narrow walk test of balance (35). They also adapted the scoring method to reduce the ceiling effects that are common in moderate to well-functioning groups. In Health ABC, the 3-year decline in lower-extremity performance assessed by the HABC-PB was consistent with clinically meaningful declines in usual gait speed and walking endurance (35).

Handgrip strength - Handgrip strength is a convenient, safe, and reliable measure of overall muscle strength. Handgrip strength of both hands will be determined separately using a hand held dynamometer with participants in the seated position with the arm resting on the table and the elbow held at approximately a right angle. The higher of two consecutive readings will be recorded as the maximum force produced with each hand. Up to 20 seconds will be allowed between measurements.

Isokinetic muscle endurance – Isokinetic muscle endurance of knee extensors and flexors will be assessed using a standard protocol on a Biodex isokinetic dynamometer. After a period of warm-up and familiarization, subjects will be asked to perform 5 maximal contractions at 240º/sec and 5 at 60º/sec. The peak torque and mean torque and its corresponding angle will be recorded. The non-dominant side will be measured. Because two tests are necessary to achieve maximal results due to the learning effect, the subjects will be tested on both day -3 and baseline. The values obtained on the baseline visit will serve as baseline.

Waist circumference (WC)

WC will be measured to the nearest millimeter with use of an anthropometric measuring tape, The measurement will be made halfway between the last rib and the iliac crest with the participant standing.

Dietary intake assessment by food frequency questionnaire

The web-based Diet History Questionnaire III (DHQ III) (past month with portions) developed by the National Cancer Institute will be administered at the baseline visit either in person or remotely (by telephone) to estimate dietary intake https://epi.grants.cancer.gov/dhq3/index.html. The DHQ III includes questions on frequency and portion for 135 food items and includes 26 dietary supplement questions. The nutrient and food group database used to analyze the DHQ III was developed using data from the National Health and Nutrition Examination Surveys (NHANES) conducted in 2007-08, 2009-10, 2011-12, and 2013-14 and provides a nutrient value by portion size and gender for each food item listed on the DHQ III. Nutrient and food group estimates in the database were derived primarily from the USDA Food and Nutrient Database for Dietary Studies, the USDA Food Patterns Equivalents Database, and the Nutrition Data System for Research (Nutrition Coordinating Center, University of Minnesota) and provides output for 219 nutrients, dietary consituents, and food groups (DHQ III Nutrient and Food Group Database. dhq.vers1005.nutdb.csv. National Cancer Institute, Epidemiology and Genomics Research Program). The Dietary Assessment Unit will administer the DHQ III and assist with the DHQ III set up, data management, and analysis.

Questionnaires

Physical Activity Scale for the Elderly (PASE) - Physical activity may potentially modify the responses to treatment. We will assess leisure, household, and occupational activity in the past 7 days either in person or remotely with use of the PASE questionnaire with 10 items and an activity score ranging from 0 to 400 (36).

SarQoL® – This is a validated 22 question health-related quality of life questionnaire for sarcopenia (37). The SarQoL® is composed of 22 questions that are rated on a 4-point Likert scale. The questionnaire is scored on 100 points. Higher score reflects a higher quality of life. Items are organized into seven domains: domain 1 'Physical and Mental Health' with 8 items; domain 2 'Locomotion' with 9 items; domain 3 'Body Composition' with 3 items; domain 4 'Functionality' with 14 items; domain 5 'Activities of daily living' with 15 items, domain 6 'Leisure activities' with 2 items, and, at last, domain 7 'Fears' with 4 items. Agreement between the test and the retest was found with an ICC of 0.91 (95% CI 0.82–0.95). It takes approximately 10 min for subjects to complete the questionnaire. The interviewer will administer the questionnaire either in person or remotely and enter the responses directly into REDCap.

Documenting study disposition

At the time that subjects complete their participation, the following information will be documented: the date, whether subject discontinued prematurely and reason, the date that subject was notified (see Study Disposition Form with instructions).

DXA

Lean tissue mass (non-fat, non-bone mass) will be measured on dual-energy absorptiometry (DXA) total body scans with a Hologic Horizon-A scanner. The root mean square precision of lean tissue mass in our laboratory is 1.26%. Non-fat, non-bone mass in the upper and lower extremity regions will be added to comprise ALM. Spine and non-dominant hip BMD will be measured on the same scanner with precision of 1.25% (spine), and 1.94% (femoral neck), and 1.60 % (total hip). A phantom provided by Hologic will be scanned weekly and the values plotted and tested for deviations.

D_3-creatine

Total body muscle mass will be assessed directly by the D_3-creatine dilution method (27). Low muscle mass measured by this method has been associated with slower walking speed, lower SPPB score, and more falls in older men (27). The D_3-creatine dilution method involves a participant ingesting a capsule containing the 60-mg dose of stable isotope-labeled creatine (D_3-creatine), and then providing a fasting morning urine sample 72–144 hours (3–6 days) later. To minimize variability in this muscle mass measurement, participants will be asked not to eat foods containing creatine or creatinine (animal products) after their mid-day meal on the day prior to these urine collections on visits 0 and 12 months. A handout will be provided to guide participants in what they should and should not eat on these two occasions. Urine D_3-creatinine, unlabeled creatinine, and creatine are measured using high performance liquid chromatography and tandem mass spectroscopy; these measures are then included in an algorithm to determine total body creatine pool size and thus skeletal muscle mass (39). The doses will be prepared by Greenpark Pharmaceuticals in Houston, TX. The urine samples will be de-identified and sent to Dr. Evan's laboratory at U.C. Berkeley for analysis. Dr. Evans will be blinded to treatment group and sequence (baseline, 12 mo) during the analyses.

Biochemical measurements

Blood will be drawn after a 12-hr overnight fast. Plasma glucose will be measured on an Olympus AU400 clinical chemistry analyzer with intra-assay CVs of 1%. IGF-1 will be measured by chemiluminescent immunoradiometric assay on an automated immunoassay system (IMMULITE 1000, Diagnostic Product Corp, LA, CA, USA) with CV of 3 – 9%. The biochemical markers of bone turnover, P1NP and CTX, were selected to assess bone formation and resorption because the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine have identified them as the most promising for clinical use (40). P1NP will be measured by competitive radioimmunoassay with RIA kits from Orion Diagnostica Uni®P1NP (Espoo, Finland) with intra- and inter-assay CVs of 5.0 and 8.1%, respectively. Serum CTX will be measured by an ELISA procedure, (Serum CrossLaps® ELISA, Immunodiagnostic Systems Inc., Fountain Hills, AZ) which employs monoclonal antibodies recognizing C-telopeptide fragments of collagen type I a1 chains containing the epitope Glu-Lys-Ala-His-Asp- Ÿ-Gly-Gly-Arg in an isomerized form, with CVs of 2-8%. IGF-1will be measured by chemiluminescent immunoradiometric assay on an automated immunoassay system, (IMMULITE 1000, Diagnostic Product Corp., Los Angeles, CA) with a CV of 3%-9%. eGFR will be calculated by the MDRD equation (41). Alanine Aminotransferase (ALT) will be measured in serum by an enzymatic kinetic procedure on a clinical chemistry analyzer (AU480 Clinical Chemistry Analyzer, Beckman Coulter, Inc., Diagnostics Division Headquarters, Brea CA 92821.) The intra- and inter- assay CVs are 4.0% and 7.0% respectively. Aspartate aminotransferase (AST) will be measured in serum by an enzymatic kinetic procedure on a clinical chemistry analyzer, (AU480 Clinical Chemistry Analyzer, Beckman Coulter, Inc., Diagnostics Division Headquarters, Brea CA 92821. The intra- and inter- assay CVs are 3.8% and 6.6% respectively. Serum 25(OH)D will be assayed by LC/MS/MS with CV of a CV of 6% in an assay calibrated to NIST standards. Serum alpha-1acidglycoprotein (AGP) will be measured by an immuneturbidmetric procedure using the AU400 clinical chemistry analyzer (Beckman Coulter, Inc., Diagnostics Division Headquarters, 250 South Kraemer Boulevard, Brea CA 92821-6232) as specified in the manufacturer's procedural documentation, with intra- and inter-assay C.V.s of 2.5% and 3.2% respectively.

G. Randomization

The Bioinformatics Specialist, Greg Matuszek, will create the one to one randomization allocation for this study using a block randomization stratified by sex. Due to the small sample size, a block size of 4 will be used. At enrollment, subjects will be randomized by a research coordinator using the randomization module in REDCap. When the subject comes for the baseline visit, a research coordinator will notify the TMC pharmacy of the treatment assignment (group A or group B). A research coordinator will pick up the pill bottle from the Pharmacy and give it to the subject. With the exception of the bioinformatics specialist, the study statistician,

and the pharmacist, all study personnel will be blinded to treatment assignment. In the event of an SAE and upon recommendation of the study physician, the bioinformatics specialist will inform the participant's primary care physician of the treatment assignment. The study physician will remain blinded.

H. Data management

Data collected during this single-site study will not leave the site. All data will be entered into electronic data capture (EDC) forms built in REDCap. Primary data records include: EDC forms completed by the research coordinator during subject interviews and physical measurements, electronic database files generated directly by the DXA bone scanning equipment, and electronic database files of biochemical screening tests generated by the Nutrition Evaluation Laboratory.

All electronic data forms will utilize data entry validation for data types and data ranges to minimize potential for error and will undergo monthly data quality checks by a member of the Biostatistics and Data Management Core Unit at the HNRCA to ensure data quality.

The study database and all source data will be maintained on the HNRCA REDCap server for a minimum of 10 years post study completion per the HNRCA data retention policy. Duplicate copies of these data will be maintained on the HNRCA disaster recovery server as well as the Bone Metabolism Laboratory network storage.

Electronic DXA data files are generated by the DXA scanner located in the Bone Metabolism Laboratory. Biochemical data are manually entered by staff of the Nutrition Evaluation Laboratory and transferred into REDCap. Original paper copies of biochemical assay results are kept in the Nutrition Evaluation Laboratory until and beyond the end of all study activity.

I. Study power

Aim 1: Muscle mass by D_3-creatine dilution –

The recently published study in Duchowny et al. (13), looked at change in muscle mass assessed by the D_3-creatine dilution method. Table 2 in the paper reports estimated mean change in D_3-Cr muscle mass over 1.6 years in 40 men (mean age 83 years), duplicated below.

Table Simple linear change

variable	mean	SD
D_3-Cr muscle mass (kg)	-1.42	1.84
weight (kg)	-0.63	2.92
D_3-Cr muscle mass/weight	-0.02	0.02

We will use SD=1.84 to represent standard deviation of change for D_3-Cr muscle mass (kg). Additionally, -1.42 kg over 1.6 years is equivalent to -0.89 kg per year (what we might expect in the placebo group).

A difference (anamorelin vs placebo) in 3 month change of 0.57 kg and 0.83 kg in appendicular LBM was observed in the two replicate studies (26). Assuming a linear rate of change, we can expect a change in muscle mass of 2.28-3.32 in 12 months. A linear rate of change is reasonable since in the safety extension of these original trials, continued response in weight change during the trial was observed out to 6 months (29).

Twelve participants per group are required to have a minimal detectable difference below 2.28 kg between anamorelin and placebo.

Aim 2: P1NP- The bone formation marker, P1NP, is the key secondary endpoint. Between-subject variation in 84-day change in P1NP was estimated from our previous clinical trial in 244 men and women age 50 and older (42). Standard deviation of 84-day change in P1NP was 0.267 nmol/L in all three treatment groups (42). With N=12 completers per group, statistical power is 82% to detect a difference of 0.32 nmol/L between placebo

and treatment, which is a 25% change. P1NP has not been measured in anamorelin treated subjects, but detecting a 25% group difference from placebo is clinically meaningful and it is plausible given that another ghrelin receptor agonist, MK-677, induced a 29% increase in another bone formation marker, serum osteocalcin, over a 2-week period (30).

J. Data analysis

Baseline subject characteristics will be tabulated and summary statistics reported for all measured outcomes at each time point.

Primary aim – The change in muscle mass over 12 months will be compared between the intervention and control groups with a two-sample Student's t-test. Should the groups be imbalanced in factors known to influence muscle mass (i.e., sex, age, HABC-PPB, physical activity level, and dietary protein intake), we will adjust for those confounders within an ANCOVA model.

Secondary aims - With 4 measurements (baseline, 2, 6, and 12 months), we will test whether the change over time is linear with use of linear models with random effects. The observed pattern of change in ALM over time will give an indication of the potential long-term benefit (or lack thereof) of anamorelin treatment. A significance level of 0.05 will be used.

The findings from secondary (and exploratory) analyses will be used for hypothesis generation, rather than to draw conclusions.

Exploratory aims – Exploratory outcomes will be analyzed as described above. For those with more than two measurements, the pattern of change will be analyzed as described above for ALM.

The primary analyses will follow intention-to-treat principles. The intent to treat population will include all subjects who had 1 or more visits after visit 0 (baseline). We will also perform per protocol analyses, in which subjects who develop clinical conditions during the study that influence the study outcomes (i.e., started treatment on an osteoporosis drug or glucocorticoids) or with selected protocol deviations will be excluded. Data will be diagnosed to evaluate validity of model assumptions. Assumptions of normality and homoscedasticity will be evaluated in these models and appropriate variable transformations will be applied if needed to satisfy model assumptions. If model assumptions do not hold for the linear mixed models, robust standard errors will be used. An exploratory per-protocol analysis will also be performed.

Safety and tolerability measures will be summarized for each treatment group based on clinical symptoms and circulating levels of glucose and ALT/AST. Adherence will be assessed by intake diary and pill counts.

K. Withdrawal from the study

Participants can go permanently "off study" only for withdrawal of consent, defined as no longer wishing to participate in all aspects of the trial. The PI may also withdraw participants for safety reasons. Participants can be off of study pills (non-adherent) but remain in the study and return for scheduled follow-up visits. Participants who are instructed by their doctor to gain weight during the study will not be withdrawn from the study.

PROTECTION OF HUMAN SUBJECTS

a. Materials and potential risks

Research material will include records made of the response to prescreening questionnaires, medical history, height, weight, blood pressure, and DXA scans on the screening visit. During the study, additional data in the form of records from study visits, responses to questionnaires and testing procedures will be obtained as described above.

b. Known potential risks to subjects

Anamorelin has the potential to cause hyperglycemia. In the two Phase 3 trials, hyperglycemia grade 1-2 (requiring treatment with diet modification or an oral agent) occurred in 5% (vs 3% in placebo) in one trial and 3% (vs <1% in placebo) in the other trial (26). Grade 3 hyperglycemia (requiring insulin) occurred in ≤ 1% of anamorelin and placebo users in both trials. Patients with uncontrolled diabetes (defined as fasting glucose ≥200 mg/dl) were excluded from participating in the published Phase 3 trials. In the 12-wk safety extension period, the incidence of drug-related AEs declined (29). Hyperglycemia occurred in 1.2% in the anamorelin group compared with none in the placebo group.

Anamorelin has the potential to cause gastrointestinal symptoms. In the two 12-wk Phase 3 trials, mild to moderate gastrointestinal complaints occurred in 6% (vs 2% in placebo) in one trial and 2% (versus <1% in placebo) in the other (26). Of the gastrointestinal complaints, nausea accounted for half to two thirds of the complaints, constipation one quarter, and vomiting 0 to one quarter. In one of the trials there were no severe gastrointestinal adverse events and in the other, severe nausea and vomiting occurred in <1% (26). In the 12-wk safety extension, nausea occurred in 0.6% of the anamorelin group, compared with 0 in the placebo group, indicating that anamorelin is suitable for long-term use (29). The risk of gastrointestinal symptoms occurring in our study subjects may be lower than in the stage 4 lung cancer patients who were often also receiving cancer treatments, but this is unknown.

Anamorelin may cause weight gain. Gains in lean mass and bone mass are the goal, thus weight gain is expected to occur. In a 12-week anamorelin trial in 174 lung cancer patients, anamorelin increased weight by 1.56 kg, in comparison with placebo and most of the gain occurred in the first 3 weeks of treatment (43). Fat mass is also expected to increase, but gains solely in fat mass have not been observed with anamorelin. To set a weight gain limit runs the risk of discontinuing those subjects who have the most favorable responses to treatment. Thus our approach is to set no weight limit but to monitor metabolic state which reflects the metabolic impact of the weight gain. Gains in lean mass are expected to improve glucose tolerance, whereas exclusive or outsized gains in fat mass would increase blood sugar. To address this and to minimize risk, we plan to exclude obese subjects and subjects taking insulin or sulfonylureas and those with fasting glucose levels on screening ≥ 150 mg/dl. To identify intra-study hyperglycemia we will monitor fasting plasma glucose at 2, 6, and 12 months.

Anamorelin at very high doses may lengthen the QT interval. A study in 60 normal adults (see Investigator brochure) revealed that a supra-therapeutic dose of 300 mg/d of anamorelin was associated with a small increase in heart rate as well as increases in the QT and PR intervals and QRS duration. *In contrast, data collected so far indicate that the therapeutic dose of anamorelin (100 mg) had no clinically meaningful effect on heart rate, QT or PR interval, or QRS duration.*

In conclusion, anamorelin was well tolerated and safe in normal adults and in advanced cancer patients most of whom were on chemotherapy and/or radiation regimens. It is highly likely to be well tolerated and safe in generally healthy community-dwelling adults in this study.

Placebo The matching placebo tablets will contain the inert substance microcrystalline cellulose that is not expected to have any adverse effect.

Blood draws Expected risks of a blood draw include slight discomfort on puncturing the skin and possible bruising in the area. Uncommon or rare risks may include phlebitis and/or scarring. The amount of blood to be drawn, 9 ml for the screening tests and 56 ml for the intra-study measures is well within the limit for research participants (maximum allowable is 500 ml over 8 weeks according to the American Red Cross).

DXA scans Radiation exposure to the region of interest from the 8 screening and intra-study DXA scans (2 spine, 2 hip, and 4 total body scans) is 60 microsieverts. This amount of radiation is about the same as one receives normally in 6 days from natural background sources.

Muscle strength and physical function testing Risk of injury from testing of physical capacity is very low. A potential risk is the risk of losing balance during the HABC-PPB testing. However, the examiner will remain on hand to help volunteers who are unsteady. At the end of the strength testing, volunteers may experience muscle tightness, soreness, cramping, or strain, but these symptoms are rare and transient.

b. Adequacy of protection against risks

Informed consent

Informed consent for both the screening and the study will be obtained from each participant by the PI or registered nurses on the Metabolic Research Unit. It will be obtained at the beginning of the screening and enrollment visits. The consenting process will take place in a private area. The PI or one of the registered nurses on the research team will go over the study information and will obtain the participants' consent. The full nature of the study (purpose, procedures, risk) will be provided to participants. Participants will be encouraged to ask questions and will be told that they can cease participating in the study at any time for any reason. Participants' consent will be documented in written form, signed by the participant and by the PI or her designee. The study protocol and consent form will have been approved by the Tufts Institutional Review Board (IRB). All personnel coming in contact with the participant will be trained and certified by the Tufts IRB in the ethical conduct of research. Subjects must be able to read and, in the view of study staff, indicate understanding of the study purpose and procedures to be eligible.

Protection against risks

To optimize intra-study safety and tolerability, we will take the following steps:

We will strictly adhere to protocol inclusion and exclusion criteria.

Blood sampling will be done by experienced nurses on the Metabolic Unit. They will use aseptic techniques.

DXA scans will be performed by DXA technicians who are experienced and certified by International Society for Clinical Densitometry and the State of MA. Should a participant's BMD of the spine or hip decline by >7% on 2 consecutive measurements, the participant will be notified and referred to his/her PCP. The participant will remain in the study.

The muscle performance measurements will be administered by an experienced research coordinator who will observe the subject during testing.

Because of the potential of anamorelin to increase fasting plasma glucose, we will monitor glucose on each visit. If it is elevated (≥ 200 mg/dl), we will repeat the measurement. If again elevated ≥ 200 mg/dl, the study pills will be discontinued. Discontinuation of study pills will be managed without unblinding the study team, and those participants will continue with study measurements (for the intent-to-treat analysis). Subjects will be asked to consume their usual dinner on the evening prior to each study visit.

Sporadic, mild increases in serum AST/ALT have been observed in subjects on anamorelin. We will monitor AST/ALT on each study visit and discontinue use of anamorelin if these levels exceed 2x the upper end of the reference range (>74 and >68 MU/ml, respectively) on repeat measurement.

In order for participants to achieve therapeutic levels of anamorelin, we will exclude subjects on CYP3A4 inducers which may increase the metabolism of anamorelin (e.g., rifampin). We will also have participants take study pills 1 hour before breakfast, as food reduces anamorelin bioavailability.

To minimize risk that anamorelin levels become supra-therapeutic, we will: a) instruct participants never to take more than one study pill per day (that is, not to 'catch up' after missing a dose); b) exclude participants taking strong CYP3A4 inhibitors (ketoconazole, clarithromycin, itraconazole, nefazodone, telithromycin); c) exclude participants taking drugs with high binding affinity to AAG because they have the potential to displace anamorelin from binding (e.g., carvedilol, chlorpromazine); d) exclude participants taking inhibitors of P-glycoprotein (e.g., verapamil, quinidine), and e) inhibitors of OATP1B3 (e.g., cyclosporine, rifampicin)

We will instruct participants to notify Dr. Dawson-Hughes of any new prescriptions (before taking the first dose) and of any changes in health status or of any hospitalizations or emergency room visits.

The pharmacokinetic profile of anamorelin has been thoroughly investigated in a number of single and repeated dose studies in healthy volunteers and patients. Main findings are illustrated in the table below (from Helsinn Therapeutics). In all studies, PK parameters were rather variable. The pharmacokinetic profile of anamorelin in cancer patients proved to be comparable to that in healthy volunteers, although variability of PK parameters was larger. We do not plan to do additional pharmacokinetics assessments in this study.

Study	Subjects	Formulation	Dose (mg)	C_{max} (ng/mL)	C_{max}/D (ng/mL) / (mg)	t_{max} (hr)	AUC_{inf} (ng·hr/mL)	AUC_{inf}/D (ng·hr/mL) / (mg)	$t_{1/2}$ (hr)	CL/F (L/h)	Vz/F (L)
HEALTHY VOLUNTEERS											
HT-ANAM-112	Healthy males and females (n=6)	50 and 100mg tablets	150.00	1281.00 (507.4)	8.54 (3.38)	0.96 (0.68)	2965.00 (319.3)	19.78 (2.12)	2.49 (0.26)	51.1 [1] (5.80)	183.2 [1] (27.00)
	Healthy males and females (n=6)	100mg tablets	200.00	1223.00 (515.4)	6.11 (2.58)	0.97 (0.44)	3071.00 (718.7)	15.36 (3.59)	2.62 (0.44)	68.30 (16.50)	259.40 (80.60)
	Healthy males and females (n=6)	100mg tablets	300.00	1977.00 (699.0)	6.59 (2.33)	0.83 (0.13)	5782.00 (1805)	19.27 (6.02)	3.11 (0.79)	56.30 (18.40)	242.50 (59.30)
	Healthy males and females (n=6)	100mg tablets	400.00	3493.00 (919.7)	8.73 (2.30)	0.71 (0.10)	8992.00 (3137)	22.51 (7.86)	3.11 (1.32)	51.70 (25.90)	211.20 (78.70)
HT-ANAM-114 [2]	Healthy males and females (n=32)	100mg tablets	100.00	901.00 (357.1)	9.01 (3.57)	0.92 (0.66)	2239.00 (1144)	22.39 (11.44)	6.35 (3.13)	57.3 [1] (29.50)	467.9 [1] (237.00)
ST-ANAM-110	Healthy males, Day 1 (n=6)	50mg tablets	150.00	879.80 (254.3)	5.87 (1.70)	0.83 (0.41)	2308.00 (706.8)	15.38 (4.71)	5.95 (1.92)	71.20 (24.70)	583.90 (186.00)
	Healthy males, Day 7 (n=6)	50mg tablets	150.00	824.00 (200.6)	5.49 (1.34)	1.54 (0.78)	2594.00 (440.0)	17.29 [3] (2.93)	7.10 (2.23)	61.70 (11.70)	650.80 (283.00)
PATIENTS											
HT-ANAM-301	Males and females NSCLC cachexia patients (n=70)	100mg tablets	100.00	674.70 (428.97)	6.75 (4.29)	1.28 (0.93)	2774.56 [3] (1450.56)	27.75 [3] (14.51)	3.05 [4]	46.13 (29.09)	202.8 [5]

1. CL/ and Vz/F for this study have been calculated post-hoc
2. Parameter values for the two control groups were pooled
3. AUCτ,ss
4. $t_{1/2}$ calculated from mean CL/F and V/F
5. V1/F+V2/F of final model

Nonclinical data indicate that the T_{max} in cardiac tissue is delayed compared with plasma T_{max} (4 hrs vs 1 hr, respectively). We will perform a screening ECG and exclude individuals with clinically meaningful abnormalities. We will also perform ECGs before and 1 and 4 hrs after the first dose of the study pills.

Stopping rules

- We will discontinue the subject's participation in the study if the QT interval exceeds 470 ms or if other clinically meaningful ECG changes occur at either 1 hr or 4-hrs post first dose (4 hrs post dose is the time when the anamorelin concentration in cardiac tissue is maximal).

- We will discontinue the subject's participation in the study in the event that he/she develops cardiac symptoms (e.g., chest pain, chest pressure, palpitations) or evidence of significant fluid retention (peripheral edema, pulmonary congestion).

- We will discontinue a subject's participation in the study if he/she is prescribed the following drugs during the study:
  CYP3A4 inhibitor (ketoconazole, clarithromycin, itraconazole, nefazodone, or telithromycin)
  Channel blocking antiarrhythmic medication (flecainide, procainamide, propafenone, quinidine)
  Drugs with high affinity to AAG (carvedilol, chlorpromazine)
  Inhibitors of P-glycoprotein (verapamil, quinidine)
  Inhibitors of OATP1B3 (cyclosporine, rifampicin)

- We will discontinue a subject's participation in the study if he/she developes glycemia defined as fasting plasma glucose ≥200 mg/dl on two consecutive measures.

Subjects developing these findings or symptoms will be referred to their physicians and these adverse events will be recorded and reported to the IRB, the NIH, and Helsinn Therapeutics. The study physician will follow up with the subject and his/her physician until symptoms have been appropriately addressed.

We will exclude subjects with eGFR < 30 ml/min.

The study will be approved by the Tufts IRB before anyone will be recruited for the study. All study personnel have been certified by the Tufts IRB to work with clinical research participants. All research activity will take place at the HNRCA. Written informed consent will be obtained from each participant.

The PI will inform the IRB of any updated information that may affect the conduct of this study or subject safety, rights, welfare or willingness to take part in the research.

Statement of compliance: The trial will be conducted in compliance with the protocol, International Council for Harmonization/Good Clinical Practice requirements (ICH/GCP), and applicable state, local and federal regulatory requirements.

Data and Safety Monitoring: The PI will ensure that each candidate meets eligibility criteria before enrollment, will closely monitor all study activities, will review and classify all adverse events, ensure that adverse events are reported in the appropriate time lines to the IRB, prepare annual IRB recertification documents. The NIH will appoint a Safety Officer who will oversee the conduct of the study. The PI will provide reports to the Safety Officer and to the NIH, with the content requested and at the requested intervals.

To ensure confidentiality of subject information: Personal information of subjects who consent to participate in the study will not be given to anyone unless the law requires it. Every effort will be made to keep subjects' information private, but this cannot be totally guaranteed. The Tufts Medical Center Institutional Review Board (IRB) or the HNRCA may check records that identify subjects. These records may include medical or research records and the signed informed consent form, which will be kept in a locked medical records room in the HNRCA Volunteer Services department. Only authorized personnel may access the record. The HNRCA Volunteer Services department is staffed during normal work hours and is locked during non-working hours. If it is necessary for a medical record to leave the admissions office, it must be signed out only by an HNRCA employee (who is identified by Tufts ID) in a log of the HNRCA ID, name of employee taking possession of the chart, and the date. The records of this study might also be reviewed to make sure all rules and guidelines were followed.

The HNRCA maintains a computerized database of participant information referred to as Protocol Manager (PM), which is a secure Oracle database that is backed up nightly. This database is centralized under the Volunteer Services Department (IRB approved protocol # 6701). Computer access to the database is provided through the Protocol Manager User Interface which was designed as an internal website ('intranet') in order to enable utilization by both PC and Mac users. An electronic gatekeeper (known as a "firewall") blocks all terminals outside of the HNRCA from gaining access. The Scientific Computing Department subscribes to Microsoft's security bulletins and appropriate security updates are applied on an ongoing basis.

Adverse events

An adverse event is defined as any untoward or unfavorable or unintended medical occurrence observed in or experienced by a participant that is not a benefit to the participant whether or not it is considered study-related by the research staff. AEs are classified by 1) seriousness, 2) expectedness, 3) relatedness, and 4) severity.

Seriousness

A **serious AE** (SAE) is any event that results in any of the following outcomes:
Death
Life-threatening condition
New inpatient hospitalization or prolongation of existing hospitalization
Persistent or significant disability or incapacity
Congenital Anomaly/Birth Defect

Requires intervention to prevent permanent impairment
Any other significant hazard that the investigators believe may require medical or surgical intervention to prevent one of the outcomes listed in this definition

A **non-serious AE** is any event that does not meet the above criteria for Serious

Expectedness

An **unanticipated problem** (UP) is defined by any adverse event, incidence, experience, or outcome that meets all of the following 3 criteria:

1. Unanticipated in terms of nature, severity, or frequency, given a) the research procedures that are described in the protocol and b) the characteristics of the study population.
2. Possibly/probably or definitely related to participation in the research.
3. Suggests that the research places participants or others at a greater risk of harm than was previously known or recognized. [This criterion is always met if the event is a SAE.]

In general, a UP may require specific action, such as modification of the protocol or consent form, suspension of enrollment of new participants, and/or suspension of study drug.

An unexpected AE is one that is not described in the medical literature, the protocol, or the informed consent documents and that does not meet the above criteria for a UP.

Relatedness

Not related (clearly not related to the intervention)
Possibly/Probably (may be related to the intervention)
Definitely related (clearly related to the intervention)

Severity refers to intensity of symptoms, degree of limitation of usual daily activities, or level of abnormality of clinical signs or laboratory parameters, as:

1. Mild. Awareness of symptoms but easily tolerated and does not interfere with usual activity. AE may or not require evaluation or therapy and may be transient
2. Moderate. This AE introduces a low level of inconvenience or concern to the participant and may interfere with daily activities but participant is able to function with minimal interference. A moderate AE may improve without any therapeutic measures or with minimal therapeutic measures.
3. Severe. A severe AE interrupts the participant's normal daily activities and generally requires systemic drug therapy, major surgery or other treatment; this AE may be incapacitating.

Reporting of adverse events

SAEs and unanticipated problems (UPs) will be reported to NIAMS, to the Safety Officer, to the IRB, and to Helsinn Therapeutics (drug-safety@helsinn.com), within 48 hrs of the PI becoming aware of them (with the exception that deaths will be reported within 24 hrs). A follow-up report of SAEs and UPs will be submitted within 5 business days.

Reports will include a narrative summary of the event, date of onset, SAE stop date, date discovered, action taken related to intervention (none, dose delayed, discontinued permanently, discontinued temporarily, other), other steps taken, expectedness (yes - described in protocol or no – not described in protocol), specific criteria met for this SAE, relationship of SAE to study intervention (not related, possibly/probably related, or definitely related)

Non-serious events will be reported to NIAMS on each progress report, to the IRB at each annual review, and to Helsinn at the end of the study.

The PI is responsible for reporting AEs. She will be assisted as needed by co-investigator, Dr. Lisa Ceglia.

Should new information become available about the safety of the intervention during the study, the PI will inform the participants by letter, phone call, or on their next visit, depending upon urgency.

Reporting to the FDA (IND 137341)
The FDA will be kept informed of the most current IRB approved protocol throughout this study so that the IND can be updated. We will send annual reports to the FDA that are linked in timing and content to the Continuing Review information sent annually to the IRB. We will send a summary of the study results to the FDA at the end of the study.

Record retention - Subjects' records will be retained for the timeframe described in the record retention policy of the "SOP – Records Retention Timeframe – Investigators."

Vulnerable Populations: This study does not include vulnerable populations.

Alternatives - Subjects may choose not to participate in the study. Their participation is voluntary. Everything done in this study is being done for research.

Withdrawal - Subjects are free to stop their participation at any time for any reason. Participants will be considered lost to follow-up if we are unable to reach them by telephone or email after trying weekly for 10 weeks. At that point, no further attempts will be made to contact them. Should subjects decide to withdraw from the study, the study measurements made before their withdrawal may be included in the scientific analysis and publication of the study results. To withdraw from the study, subjects can contact Dr. Dawson-Hughes at 617-556-3265. Dr. Dawson-Hughes may also end a subject's participation in the study at any time in order to protect his/her safety or to maintain the smooth conduct of the study.

c. Potential benefits to subjects

This study is not designed to benefit individual subjects. Screening lab results will be made available to the subjects. Subjects will have the option to receive copies of their screening spine and hip DXA scans two to three weeks after their visit. Intra-study laboratory tests will be made for research only and will not be shared with participants. If any adverse medical conditions are discovered during the study, subjects will be notified and referred to their doctor.

Risk/benefit assessment
The risks incurred are minimal relative to the potential benefit of gaining information about one's health.

Payment plan
Subjects will be paid $25 for the screening visit. Each subject who completes the study will be paid an additional $700 for transportation and miscellaneous expenses. Of this, $200 will be given after the baseline visits, $100 will be given after the 2-month and the 6-month visits, and $300 will be given after the 12-month visits. Participants asked to return for safety laboratory assessment will receive $25 for each extra visit. If a subject needs to remain in the study for up to 10 extra days for any reason (such as snow days, unrelated minor illness, unanticipated schedule change), he/she will continue in the study, however the stipend will not be increased. If a subject drops out of the study or is obliged to skip any of the visits, his/her payment will be pro-rated to the portion of the protocol that he/she completed according to the payment schedule described above. The HNRCA will mail a check to participants about two to three weeks after the visits described above.

Reimbursement for research related injury
Emergency medical treatment will be given to a subject who is hurt or gets sick as a direct result of being in this study. The subject or his/her insurance carrier will be required to pay for any such medical care. There is no money to pay for treatment if a subject gets hurt or sick as part of this study. Any needed medical care is

available at the usual cost. All needed facilities, emergency treatment, and professional services are available to the subjects, just as they are to the general public. The institution will not pay for treatment if a subject becomes ill or injured as part of this study.

Collection and storage of human biological specimens

Upon completion of the study and data analysis, any remaining blood samples will be discarded. In addition to testing during the study, blood and urine will be archived for future measurements (i.e., batched measurements at the end of all subject visits). All samples will be used for the purpose of this study only and will be discarded at the end of the study. Participants will be issued an individually identifiable number linking records, specimens and data. Urine samples will be de-identified before they are sent to Dr. Evans at UC Berkeley for D_3-creatine analysis.

Vulnerable populations – This study does not include vulnerable populations.

ClinicalTrials.gov – This study will be registered in ClinicalTrials.gov at least 21 days before the first subject is enrolled.

Importance of the knowledge – The goal of this study is to identify a potential treatment for low muscle mass and low bone mass. If effective in this small study, anamorelin may warrant further investigation.

Risk/importance assessment – The potential benefits of the proposed research study outweigh the potential risks to participants. The potential for serious adverse events is small and the potential benefit to society is high enough to justify conducting this study.

Study sponsorship

This study is funded by a research grant from the National Institutes of Health (1 R21 AR074138-01A1). Study pills will be provided by Helsinn Therapeutics. Study activities will take place at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University. All study documents will be approved by the Tufts IRB before the study starts.

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