Study Protocol for the Parent Study:
Title: Long-term Effects of Weight Loss and Supplemental Protein on Physical Function
NCT03074643
Date: 08/02/2022

***Note the IRB-approved study protocol for the parent study above was utilized for this ancillary study:**
Title: Utilizing Protein During Weight Loss to Impact Physical Function and Bone
NCT03819478

Scientific Study Title: Long-term effects of weight loss and supplemental protein on physical function

Lay Title: UPLIFT (Utilizing Protein During Weight Loss to Impact Physical Function)

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Sponsor or funding source: NIH/NIA

Background, Rationale and Context

Obesity exacerbates age-related declines in physical function and is a strong determinant of mobility disability.[1-3] One-third of older adults are obese;[4] thus, there is an urgent need to identify effective therapies that prevent obesity-related declines in physical function. The loss of lean mass with age contributes to age-related declines in physical function.[5-7] Since approximately one-fourth of lost weight is comprised of lean mass,[8-11] current weight loss recommendations regarding macronutrient composition – and protein in particular – during caloric restriction may exacerbate the loss of lean mass in older adults, further compromising physical function over the long-term. Moreover, studies suggest that older adults need more protein to elicit the same stimulatory effect on muscle protein synthesis and maintain lean mass than younger adults.[12-16] Thus, recommendations for effective weight loss treatments that preserve lean mass and optimize function in obese older adults may differ from those for younger adults. Additionally, recent *Guidelines (2013) for the Management of Overweight and Obesity in Adults* stated "there is a need for further research to understand the most appropriate strategies and prescriptions for weight loss [in] older adults."[17]

Limited evidence in older adults shows that weight loss improves physical function while in the weight-reduced state.[8;18-24] However, most people are not successful at long-term maintenance of weight loss.[25] Whether improvements in physical function persist is unknown, particularly if weight regain occurs. We and others have shown that weight regain after weight loss resulted in greater regain of fat relative to lean mass a year after the intervention.[26;27] Consequently, weight loss and subsequent regain may lead to even less relative lean mass and worse physical function. This problem highlights the need to identify a strategy that preserves lean mass during weight loss and promotes the deposition of lean mass following weight loss. Similar to studies in younger adults,[28] we previously found that a higher protein diet during caloric restriction may be a key determinant for retaining lean mass during weight loss in postmenopausal women.[10;29] A higher protein diet after weight loss may also enhance weight loss maintenance by reducing overall weight regain, in part, by favoring regain of lean mass over fat.[30] Thus, the potential benefits of a higher protein diet during and subsequent to weight loss in obese older adults should translate into even greater improvements in physical function over both the short- and long-term; however, this needs to be definitively tested.

Objectives

Our **primary goal** is to determine the effect of a higher protein (1.2 g/kg body wt/d; ~30% of energy) / lower carbohydrate (CHO; 40-45% of energy) diet during a 6-month weight loss intervention in obese older adults on physical function compared with an isocaloric lower protein (current RDA of 0.8 g/kg body wt/d; 15-20% of energy) / higher CHO (55-60% of energy) diet, and whether continuing a higher protein / lower CHO diet following weight loss results in better maintenance of physical function. This will be accomplished with an 18-month randomized trial in 225 obese (BMI 30-45 kg/m^2 or 27-30 kg/m^2 with 1 additional risk factor), older (65-85 years) men and women at risk for disability (expSPPB <2.50). All participants will undergo a 6-month weight loss intervention of caloric restriction and exercise followed by 12 months of follow-up with randomization to one of three groups (n=75/group): 1) Lower protein / higher CHO diet for the 6-month weight loss and 12-month follow-up phases (RecProt); 2) Higher protein / lower CHO diet for the 6-month weight loss phase only (6-mo HiProt); or 3) Higher protein / lower CHO diet for the 6-month weight loss and 12-month follow-up phases (18-mo HiProt).

Primary Aim: To determine the effects of a higher protein / lower CHO diet vs. lower protein / higher CHO diet during a 6-month weight loss intervention and 12 months of follow-up on 18-month physical function assessed by the expanded Short Physical Performance Battery (primary outcome) and muscle strength.

Hypothesis: Participants randomized to a higher protein diet during weight loss and follow-up (18-mo HiProt) will have greater improvements in physical function than participants randomized to a higher protein diet during weight loss only (6-mo HiProt), followed by those randomized to a lower protein diet (RecProt).

Secondary Aim: To determine the effects of a higher protein / lower CHO diet vs. lower protein / higher CHO diet during a 6-month weight loss intervention and 12 months of follow-up on 18-month body weight and body composition.

Hypothesis: Participants randomized to a higher protein diet during weight loss and follow-up (18-mo HiProt) will have better weight loss maintenance, lower fat-to-lean mass ratio, greater thigh muscle volume, and lower inter-muscular adipose tissue than participants randomized to a higher protein diet during weight loss only (6-mo HiProt), followed by those randomized to a lower protein diet (RecProt).

We will also assess the effects of a higher protein diet on: 1) 6-month changes in physical function, weight, and body composition; 2) 6- and 18-month changes in bone mineral density; and 3) 6- and 18-month changes in cardiometabolic risk factors (e.g., glucose and lipids); and 4) whether the effects of a higher protein diet on lean mass mediates the effects on physical function at 6- and 18-months.

Methods and Measures

Study Overview:

We will use a 3-group design in 225 obese (BMI 27-30kg/m^2 with 1 risk factor or BMI 30-45 kg/m^2), older (65-79 years) men and women at risk for disability (expSPPB <2.50) who will undergo a 6-month weight loss intervention followed by a 12-month follow-up phase to test our overall hypothesis that a higher protein (1.2 g/kg body wt/d) / lower CHO diet during a 6-month weight loss intervention improves physical function compared with an isocaloric lower protein (the current RDA, 0.8 g/kg body wt/d) / higher CHO diet and continuing a higher protein / lower CHO diet for 12-months following weight loss will result in better maintenance of improved physical function. All participants will undergo a 6-month weight loss intervention involving caloric restriction and supervised exercise followed by 12 months of follow-up with randomization to one of three groups (n=75/group): 1) Lower protein / higher CHO diet for the 6-month weight loss and 12-month follow-up phases (RecProt); 2) Higher protein / lower CHO diet for the 6-month weight loss phase only (6-mo HiProt); or 3) Higher protein / lower CHO diet for the 6-month weight loss and 12-month follow-up phases (18-mo HiProt). Physical function (primary aim) will be assessed by the expanded Short Physical Performance Battery (expSPPB) and muscle strength and body composition (secondary aim) assessed by dual-energy X-ray absorptiometry (DXA) and computed tomography (CT) at baseline, 6- and 18-months. At the same time points, we will also measure bone mineral density (DXA) and cardiometabolic risk factors.

Recruitment of study participants:

We plan to recruit, screen, and enroll 225 participants in Years 1-3 (~1-2 per week). Our recruitment goals include: 30% male and 25% minority. We will target men and women ≥65 years who live in the Piedmont Triad, including the Greensboro metropolitan area (a 20-30 min drive from the Medical Center). We will use community-based recruitment strategies developed by the Clinical Research Core of the WFU Pepper Center, including direct mail, newspaper advertisements, and cross-study referrals from other on-going studies. **Specific inclusion/exclusion criteria (see Table 1) are in place to eliminate those that may be adversely affected by the interventions or that are not able to comply with the interventions**. To reduce the potential adverse effects of WL, we will exclude individuals that are non-obese, those with recent WL, those who have osteoporosis, and/or anyone on medications that may affect bone density. We also exclude anyone who cannot walk unassisted.

Participant screening and randomization:

All individuals who respond to our recruitment strategies will call a toll-free phone number and a recruiter will describe the study and perform a brief screen for general eligibility (see **Table 1**). They will be asked about their age, weight, height, current physical activity habits, body weight in the past year, smoking history, medical history, and current medications. All participants must conform to the inclusion/exclusion criteria.

Individuals who pass the telephone screening will be scheduled for an in-clinic screening visit (SV1) which is conducted in the early morning following an overnight (8 hr) fast. Before any data collection, all participants will provide written informed consent and complete a HIPAA authorization form in accordance with the WFSM IRB policies. At this visit, weight and height will be measured to calculate BMI. After confirmation of meeting the

BMI inclusion criteria, individuals will undergo further screening including the expanded Short Physical Performance Battery (expSPPB), a cognitive screen (Montreal Cognitive Assessment, MoCA[31]), physical activity assessment (the Community Healthy Activities Model Program for Seniors [CHAMPS] questionnaire[32]) to ensure no regular participation in resistance exercise or high-volume aerobic training in the past 6 months, a depressive symptom screening test (Center for Epidemiological Studies-Depression, CES-D[33]), a weight loss readiness questionnaire, and, if still eligible, a medical history, a comprehensive review of medications, blood pressure, and a fasting blood draw for a metabolic screening panel and complete blood count (CBC) to exclude those with recent history of coronary heart disease, cancer, liver or renal disease, severe pulmonary disease, gross physical impairment, uncontrolled hypertension, uncontrolled diabetes, or any contraindications to exercise. Participants will also be given a 4-day food record to complete. If still eligible, a second in-clinic screening visit will be scheduled for a GXT (to rule out any contraindications for supervised exercise) and DXA scan (to rule out osteoporosis) and to collect the 4-day food record. Participants will also do a taste test of the protein and carbohydrate supplements to ensure that they are willing to consume the product during the intervention and complete a perceived weight loss readiness questionnaire.

Table 1. Inclusion/exclusion Criteria

Criteria	Inclusion	Exclusion	Assessment
Age	65-85 years		Self-report
Obesity status	BMI: 30-45 kg/m^2 or BMI 27.0 – <30.0 **AND** at least **ONE** of the following risk factors: 1) elevated waist circumference (>35 inches in women, >40 inches in men) 2) diabetes, 3) hypertension, 4) dyslipidemia, 5) or other obesity-related comorbidities: clinically manifest coronary artery disease [e.g., history of MI, angina pectoris, coronary artery surgery, coronary artery procedures (e.g., angioplasty) *if not within the past year*], other atherosclerotic disease [e.g., peripheral arterial disease, abdominal aortic aneurysm, symptomatic carotid artery disease *if not within the past year*], sleep apnea, or osteoarthritis of the knee or hip.		Measured on scale 1)Measured with tape measure 2-4) Self-report and treatment 5) Self-report
Weight loss		Weight loss (≥5%) in past 6 months	Self-report
Physical activity status	No regular (moderate intensity or greater) resistance training and/or aerobic exercise (>20 mins/d) for past 6 months		Self-report
Functional status	expSPPB <2.50	Dependent on cane or walker	expSPPB / self-report
Cognitive status		Cognitive impairment (MoCA score <22)	Questionnaire
Orthopedic status	No contraindications for safe and optimal participation in exercise training	Severe arthritis, or other musculoskeletal disorder Joint replacement or other orthopedic surgery in past 6 months	Self-report
Co-morbidity/health status	Approved for participation by Medical Director (Dr. Lyles)	Uncontrolled resting hypertension (>160/90 mmHg); Uncontrolled diabetes (HbA1c ≥8.0%) Current or recent past (within 1 year) severe symptomatic heart disease,	BP measurement HbA1c blood test Self-report

Table 1. Inclusion/exclusion Criteria			
Criteria	**Inclusion**	**Exclusion**	**Assessment**
		uncontrolled angina, stroke, chronic respiratory disease requiring oxygen; uncontrolled endocrine/metabolic disease; neurological or hematological disease; cancer requiring treatment in past year, except non-melanoma skin cancers; liver or renal disease; or clinically evident edema	
		Unstable Severe Depression	Questionnaire (CES-D)
		Serious conduction disorder, uncontrolled arrhythmia, or new Q waves or ST-segment depressions (>3 mm at rest or ≥2 mm with exercise)	GXT
		Abnormal kidney function (eGFR <30 based on serum creatinine, age, gender, and race)	Metabolic panel
		Anemia (Hb<13 g/dL in men; <12 g/dL in women)	CBC
		Drug abuse or excessive alcohol use (>7 drinks/week women; >14 drinks/week men)	Self-report
		Use of any tobacco or nicotine products in the past year	Self-report
		Osteoporosis (T-score $\leq$ -2.5 on hip or spine scan)	DXA
Medication / supplement use		Regular use of growth hormone, anabolic steroids/hormones, sex steroids (testosterone; estrogen – oral or patch) or corticosteroids (medications that can affect muscle mass/strength and/or bone)	Self-report and medication inventory
		Osteoporosis medication	
		Protein supplements (and unwilling to stop using for duration of study)	
		Weight loss medications or procedures	
Research participation	Willing to provide informed consent	Current participation in another intervention study	Self-report
	Agree to all study procedures and assessments		
	Willing to consume protein/CHO supplements for up to 18 months		Taste test
	Able to provide own transportation to study visits and intervention sessions		

Individuals who meet all eligibility criteria and provide informed consent will be randomized to one of the three intervention groups (following all baseline testing) using a web-based randomization scheme (developed by Dr. Leng) with blocking stratified by sex. Block size and the randomization sequences will be unknown to the research staff. Treatment assignment will be generated using a program that the study coordinator will access from her PC via the study website. Individuals with abnormal results at screening will be referred to their physicians.

Study Interventions

6-month weight loss intervention:

Caloric restriction and behavior modification/nutrition education: All participants will undergo a dietary weight loss intervention designed to elicit behavioral changes leading to decreased caloric intake sufficient to yield a ~10% loss of initial body mass. Recommended weight loss interventions for obese older adults include moderate caloric restriction (CR) to elicit a weight loss of 0.5-1 kg/wk.[1;34-36] Thus, the 6-month weight loss

intervention is designed to elicit ~0.5 kg/wk weight loss through a CR level of -500 kcal/d, plus an exercise prescription that meets current American Heart Association (AHA) and American College of Sports Medicine (ACSM) physical activity recommendations for older adults (described below).[37] With a calorie deficit of 500 kcal/d, we expect total weight loss of ~10.0 kg. The reduced calorie level is derived by subtracting 500 from each persons estimated daily energy needs for weight maintenance. Individual energy needs are calculated from the direct measurement of resting metabolic rate and applying an activity factor based on a description of their daily activities (typically 1.3 for sedentary persons). No woman will be recommended <1100 kcals/d and no man <1300 kcals/d.

The weight loss intervention will incorporate nutrition education (via group and individual meetings with the study dietitian), self-monitoring skills, cognitive-behavioral strategies for promoting lifestyle behavior modifications, and planned meals. Behavioral and educational group and individual sessions will be held weekly in a 3:1 ratio (3 group and 1 individual session per month). Group sessions will be held in-person in small groups (maximum of 6 participants per group) to maintain physical distancing (with the option to go entirely remotely via WebEx or phone if the conditions arise – e.g., increase in COVID-19 community spread, new restrictions on in-person research are put in place, and/or if stay at home orders are put back in place). Individual sessions will be held in-person with the option of remote sessions (via WebEx or phone) if the conditions arise. The diet intervention will be based on participants choosing and preparing their own meals from the meal plans. Sessions will be led by the study dietitian and/or interventionist with expertise in behavior therapy with older adults. All participants will be instructed in behavior modification with advice on food selection, portion size, relapse prevention, and self-monitoring techniques. Emphasis will be placed on following the menus provided. The behavioral sessions will focus on awareness of changing eating habits to lower caloric intake. Factual information regarding what food changes to make, how to make them, and why they are important will be explained. Each group session will include problem solving. During the individual sessions, the study dietitian will review individual progress, solve problems, answer questions, and set goals. Examples of topics to be covered include calorie balance, emotional eating, eating out, self-monitoring, reacting to environmental and social cues, portion control, and relapse prevention. All participants will be taught self-monitoring techniques for diet and body weight. They will be asked to track their daily intake of all foods and beverages in a log book provided to them, and given feedback on their log books at each session.

Meal plans will be developed based on estimated calorie levels in 100 calorie increments (1100 calories, 1200 calories, 1300 calories, etc) to attain the weight loss goal. Each meal plan will have up to 7 interchangeable choices at each meal (breakfast, lunch, dinner) and snack (midday and evening). At each meal, participants within a given calorie level will select foods from the list to prepare and consume. There will also be choices that allow for eating meals at restaurants that meet the specified guidelines. Meals will differ between calorie levels in portion size and types of foods. In addition, there will be different selections for those on the higher protein / lower CHO diet vs. the lower protein / higher CHO diet (see below). The food plan will allow for individual preferences for various food items, and the study dietitian will work with participants to tailor the diet to individual needs. Participants will be advised to take calcium (500-1000 mg/d) and vitamin D (800 IU/d) supplements to ensure adequate intake of these nutrients.

Body weight will be monitored and recorded weekly. Participant logs of everything they eat or drink will be reviewed weekly by the study dietitian to verify compliance to the diet. If a participant is not meeting weight loss goals (~0.5 kg/wk), calorie intakes will be individually modified to produce the desired rate of weight loss. If it is evident, either through diet logs or inadequate weight loss (~0.5 kg/wk), that participants are not compliant, individual counseling sessions with the study dietitian will be held to improve compliance. For participants who have difficulty in achieving the weight-loss and/or protein intake goals, we will use a behavioral “toolbox” that includes frequent individual behavioral counseling sessions, prescription of meal replacements, incentives, home-assessments, or other items deemed appropriate. Diet plans may be altered to restrict snacks and/or the size of meals to meet weight loss goals.

Macronutrient distribution: Those in the higher protein / lower CHO diet groups (6-mo HiProt and 18-mo HiProt) will be prescribed a diet containing more protein than those in the lower protein (the current RDA) / higher CHO diet group (RecProt). To ensure the diets are isocaloric, this protein will replace part of the carbohydrate prescribed in the lower protein / higher CHO diet group. Individuals assigned to the lower protein / higher CHO diet will have a targeted protein intake of 0.8 g/kg body wt/d (~15-20% of energy), 25-30% of energy from fat (<10% from saturated fat), and 55-60% of energy from carbohydrates. The higher protein / lower CHO diet groups will have a targeted protein intake of 1.2 g/kg body wt/d (~30% of energy) with a

reduction in CHO to 40-45% of energy. *Both the higher and lower protein diet groups fall within the Dietary Reference Intakes for macronutrients including protein (10-35% of energy).*[38] Fat intake will remain similar between groups while CHO will fluctuate to compensate for differences in energy from protein between groups. Participants in the higher protein / lower CHO diet groups will consume a protein supplement daily (NutraBio™ 100% Whey Protein Isolate) which will be provided throughout the study. The supplement will provide ~50 g of high quality protein containing 22.6 g of essential amino acids, 10.7 g branch chain amino acids consisting of 5.2 g of leucine, 3.0 g of isoleucine, 2.5 g of valine, and 200 calories. Since protein intake tends to be skewed towards higher amounts at the evening meal[39] and data suggest a protein threshold of 25-30 g protein containing ~2.5-2.8 g of leucine/meal optimizes protein synthesis,[40-42] participants will be instructed to consume 25 g (1 scoop) of the protein supplement twice daily with their morning and midday meal/afternoon snack. Participants in the lower protein / higher CHO diet group will consume a carbohydrate supplement daily (NutraBio CarboMax; provided throughout the study) which will provide ~50 g of maltodextrin and 200 calories to maintain blinding during the 6 month weight loss intervention.

At the first individual meeting with the study dietitian following randomization, the study dietitian will provide each participant with a 6-month supply of protein or carbohydrate supplement and provide oral and written instructions for using the study supplement. The study dietitian will also provide a 6-mo supply of protein for those in the 18-mo HiProt group at the end of the 6 month weight loss intervention and at the 6-mo post-weight loss follow-up visit.

Exercise Intervention: All participants will be expected to participate in a supervised, center-based exercise program involving moderate-intensity aerobic exercise (e.g., treadmill walking) 3 days/wk during the first 6 weeks of the 6-month weight loss intervention in accordance with the AHA and ACSM physical activity recommendations for older adults.[37] All participant supervised, center-based exercise sessions will be scheduled in advance to ensure that physical distancing can be maintained (maximum of 5 participants per session). Supervised exercise sessions will occur in our research exercise facility under the direction of an exercise interventionist to minimize individual variability in compliance and progression. Injuries will be minimized by a feasible progression of exercise duration and careful monitoring of proper footwear and stretching exercises. Each exercise session will begin and end with 3-5 min of walking at slow pace and light stretching. The aerobic training program will progress from 15-20 min at 50% heart rate reserve (HRR; based on age-predicted maximal heart rate) the 1st week to 40 min at 65-70% HRR by the end of the 6th week. Heart rate (HR) will be measured before each exercise session and at least two HR readings (measured by Polar heart rate monitors) will be taken during aerobic exercise. Treadmill speed and grade will be adjusted as necessary to ensure that participants exercise at their prescribed exercise intensity. Treadmill speed and grade, heart rate, exercise duration, and amount of energy expended will be recorded for each exercise session. After the first 6 weeks of the 6-month weight loss intervention, participants will continue center-based exercise 1-2 days/wk and exercise on their own 1-2 days/wk for a total of 3 days/wk combined center- and home-based exercise (with the option of transitioning to all home-based exercise if the conditions arise – e.g., increase in COVID-19 community-spread, new restrictions on in-person research are put in place, and/or if stay at home orders are put back in place). Participants will be taught how to rate their perceived exertion so that their home-based exercise mirrors that done during their supervised, center-based exercise sessions (target RPE between 13 and 15) and given tips on how to exercise safely on their own. Participants will be asked to keep exercise logs detailing the date, type, duration and their rating of perceived exertion for each home-based exercise session. Compliance to the exercise prescription (attendance/ frequency, intensity and duration) will be monitored. The goal for attendance at center- and home-based exercise sessions is >80% and participants who miss >3 sessions per month will be counseled to improve exercise attendance.

<u>12-month follow-up:</u> At the end of the 6-month weight loss intervention, the study dietitian will meet individually with all participants to devise a weight maintenance strategy, including sample meal plans. Participants will be encouraged to use relapse-prevention techniques, e.g., managing the environment, adjusting appropriate goal-based daily energy needs, and continuing self-regulatory skills. At the 6-month post-weight loss intervention follow-up visit, participants will again meet individually with the study dietitian, review their weight maintenance goals and strategies, and make modifications as needed. Participants randomized to a higher protein / lower CHO diet during the 12-month follow-up period (18-mo HiProt) will be provided with a protein supplement (see above) to consume daily and counseled on how to include the protein supplement in their maintenance meal plan. Participants who have difficulty consuming the protein supplement will be given the option to reduce the

frequency of the protein supplement by increasing dietary protein from food sources. Group seminars on health-related topics will be held quarterly to keep participants engaged in the study.

Schedule and organization of assessment visits

All assessments will be conducted during 7 visits (two screening, two baseline, one 6-month post-weight loss intervention, one 12-month follow-up, and one 18-month follow-up visits; **Table 2**). The nature, purpose, and risks of all tests will be explained to participants prior to obtaining their written consent and prior to each test.

Table 2. Assessment timeline Visit Code	SV1	SV2	BV1	BV2		FV1/2 (6-mo)		FV3 (12-mo)		FV4/5 (18-mo)
Informed consent/HIPAA form, weight, height, waist circumference, blood pressure, expanded SPPB, demographics, cognitive screen (MoCA), depression screen (CES-D), weight loss readiness questionnaire, fasting screening blood draw (CMP and CBC), CHAMPS physical activity questionnaire, medical history and medication review, instructions on how to keep a 4d food record	X				6-month intervention		6-month follow-up		12-month follow-up	
Collection of 4d food record		X								
Review Inclusion/Exclusion criteria	X	X								
Protein/CHO taste test		X								
Medication review		X	X	X		X		X		X
Weight		X	X	X		X		X		X
Blood pressure				X		X		X		X
RMR				X		X				(X)***
Arterial stiffness				X		X				(X)***
GXT and Perceived Fatigue		X				(X)**				
Expanded SPPB*, leg strength, grip strength, and 400-m walk			X			X		X		X
24-hr dietary recalls			X	X		X				X
Fasting blood draw				X		X				X
Anthropometrics		X				X				X
DXA scans		X				X				X
CT scans			X			X				X
CHAMPS physical activity questionnaire						X		X		X
MAT-sf			X			X		X		X
CES-D						X		X		X
SF-36			X			X		X		X
DSST			X			X				X
Fatigue questionnaire (FACIT)			X			X		X		X
Instructions for 24-hr urine sample collection†			X			X				X
Return 24-hr urine sample†				X		X				X

* Expanded SPPB at FV1/2, FV3, and FV4/5 only.

† Collected from ppts after FV1/2; delivered to ppts prior to FV4/5.

** Dropped after COVID19 in-person research pause to allow us to combine FV1 & FV2 visits

*** Dropped after COVID19 in-person research pause to allow us to combine FV4 & FV5 visits

All assessments will take place by study staff blinded to the participant's treatment assignment. To ensure that staff remain blinded, participants will be asked not to discuss their intervention with the assessor. Additionally, the staff member will remind the participants not to tell them which intervention group they have been assigned to.

The first screening visit (SV1) and the resting metabolic rate (RMR; BV2 and FV1/2 (6-mo)) and fasting blood draw visits (BV2, FV1/2 (6-mo), and FV4/5 (18-mo)) will occur in the early morning after an 8 hour fast. After a blood draw for the metabolic screening panel and CBC (SV1), RMR (BV2 and FV1/2), and fasting blood (BV2, FV1/2, and FV4/5), participants will be given a light breakfast. BV1 and BV2 will occur approximately one week apart. The follow-up visits will occur (1) after the 6-month intervention (FV1/2; weeks 27-32), (2) 6 months post-intervention (FV3; weeks 53-56), and (3) 12 months post-intervention (FV4/5; weeks 79-84).

The **first screening visit (SV1)** will be conducted in the morning following an 8 hour overnight fast for measurement of a complete metabolic panel and CBC for screening purposes. After blood pressure, pulse, height and weight are collected and the blood draw completed, participants will be given a snack and continue with the remainder of the visit. First, the expanded Short Physical Performance Battery (expSPPB)[43] will be administered and scored. If still eligible, participants will then undergo a cognitive screen (assessed using the Montreal Cognitive Assessment, MoCA[16]), a depression screen (assessed using the Center for Epidemiological Studies-Depression (CES-D)), a weight loss readiness questionnaire, and a medical history and review of medications and dietary supplements. The CHAMPS Physical Activity Questionnaire for Older Adults[32] will be administered to estimate physical activity in a typical week over the past 4 weeks. Demographics will also be collected. Potentially eligible participants will be given instructions on how to keep a food diary, and asked to record four days of food intake prior to the second screening visit. Following the first screening visit, the study coordinator will check the lab results to determine if the participant is still eligible based on blood work. If the participant is eligible, they will be notified and scheduled for a second screening visit. If the participant is not eligible, they will be notified and given a copy of their screening blood work.

At the **second screening visit (SV2)**, participants will undergo an assessment of Perceived Fatigue on the treadmill as a warm-up to acclimate participants to the treadmill. Then participants will do a graded exercise stress test (VO_2 max) using a Ramp protocol[44] to exclude those with exercise-induced ischemia. All participants must be free of any contraindications for participation in an exercise program according to the American College of Sports Medicine criteria.[44] These criteria will eliminate those with conditions that may affect their ability to complete a 6-month exercise program. Whole-body, AP spine, and hip DXA scans will be conducted to exclude persons with osteoporosis. Any individual with abnormal test results at their screening visit will be referred to their physician. The participant will have anthropometric measurements taken at their waist, hip and thigh. Food records will be collected and evaluated to insure participants will be willing to record dietary intake during the 6-mo weight loss phase and provide baseline dietary preferences to the study RD. Participants will be given a taste test of the protein and carbohydrate supplements to ensure they are willing to consume them for the duration of the study. Participants who are eligible to participate in the study will be randomized to one of the 3 study intervention groups (following all baseline testing) using a web-based randomization scheme (from Dr. Leng) with blocking stratified by gender.

At the **first baseline visit 1 (BV1)**, weight, lower extremity muscle strength (Biodex dynamometer), grip strength, and 400-m walk time will be measured. Any changes to medical history and current medications and dietary supplements will be reviewed. Then the Mobility Assessment Tool for Disability – short form (MAT-sf) to assess difficulty performing daily tasks, the Short Form 36 (SF-36) to assess quality of life, the Digit Symbol Substitution Test (DSST) to assess attention and psychomotor speed, and the FACIT questionnaire to assess fatigue will be administered. The participant will also have a series of CT scans (abdomen, hip and thigh) at this visit. Participants will be given a 24-hr urine collection bottle and instructions for collecting urine. Participants will be scheduled for their BV2 visit approximately one week later.

Baseline visit 2 (BV2) will take place approximately 1 week after the BV1 visit in the morning (after an 8 hour overnight fast). Participants will have their weight and blood pressure measured. Then participants will have their resting metabolic rate (RMR) assessed for the purpose of estimating each person's baseline calorie level. Participants will also undergo tests to assess arterial stiffness, including aortic augmentation index (AIx) and arterial wave reflection magnitude. Participants will then have blood drawn for glucose, insulin, HbA1c, lipids, and storage. The first of three 24-hr dietary recalls will be administered to introduce and train the participant for the procedures used in subsequent 24-hr dietary recalls done by phone (in the event that scheduling precludes

administration of the first 24-hr dietary recall in person, the participant will be given written instructions and the first 24-hr dietary recall administered by phone). Two additional 24-hr diet recalls will be administered by phone following BV2 but prior to the participant's first intervention visit. (24-hr diet recalls may be started at BV1 for some participants to ensure they are completed prior to the start of intervention.) Following all fasting measures, participants will be given a snack. Medical history and current medications and dietary supplements will be reviewed. The 24-hour urine sample will be collected to measure urinary nitrogen and creatinine for estimating dietary protein intake and for storage.

Randomization/first intervention visit. Due to the group nature of the diet classes, participants will be randomized in waves with all participants starting intervention in a given week. The study interventionist will check to see which intervention group the participant is randomized to at their first meeting with each eligible participant during the first week of each wave's intervention.

The **6-month follow-up visit (FV1/2)** will take place following week 26 of the intervention in the morning after an 8 hour overnight fast. Participants will have their weight and blood pressure measured. Then participants will have their resting metabolic rate (RMR) measured for the purpose of estimating each person's weight maintenance calorie level. The participant will also undergo tests to assess arterial stiffness. Blood will be drawn for glucose, insulin, HbA1c, lipids, CMP and CBC, and storage. Participants will then be provided with a snack. Any changes to medical history and current medications and dietary supplements will be reviewed. Whole-body, AP spine, and hip DXA scans will be done. The participant will have anthropometric measurements taken at their waist, hip and thigh. Lower extremity muscle strength (Biodex dynamometer), grip strength, physical performance (expSPPB), and 400-m walk time will be measured. The MAT-sf and DSST will be administered. The CHAMPS Physical Activity Questionnaire, CES-D, SF-36, and FACIT questionnaires will be administered at the visit time permitting or by phone after the completion of the in-person visit. A series of CT scans (abdomen, hip and thigh) will be done. Participants will be given the 24-hr urine collection bottle and instructions for collecting urine to measure urinary nitrogen and creatinine for estimating dietary protein intake and for storage. Three 24-hr diet recalls will be administered by phone during the FV1/2 visit window. . Participants will schedule to drop off their 24-hour urine sample following their FV1/2 visit

Monthly phone calls: Participants will be called once a month during the 12-mo follow-up to ensure those that are in the 18-mo HiProt are continuing to consume the protein supplement. All participants will be asked about any health changes, injuries, etc., that may have occurred and any events recorded using the Adverse Event Form.

The **12-month follow-up visit (FV3)** will take place following week 52 of the intervention. Participants will have their weight and blood pressure measured. Lower extremity muscle strength, grip strength, physical performance (expSPPB), and 400-m walk time will be measured. The CHAMPS Physical Activity Questionnaire, MAT-sf, CES-D, SF-36, and FACIT questionnaires will be administered. Medical history and current medications and dietary supplements will be reviewed.

The **18-month follow-up visit (FV4/5)** will take place following week 78 in the morning (after an 8 hour overnight fast). Participants will have their weight and blood pressure measured. Blood will be drawn for glucose, insulin, HbA1c, lipids, CMP and CBC, and storage. Participants will then be provided with a snack. Any changes to medical history and current medications and dietary supplements will be reviewed. Whole-body, AP spine, and hip DXA scans will be done. The participant will have anthropometric measurements taken at their waist, hip and thigh. Lower extremity muscle strength (Biodex dynamometer), grip strength, physical performance (expSPPB), and 400-m walk time will be measured. The MAT-sf and DSST will be administered. The CHAMPS Physical Activity Questionnaire, CES-D, SF-36, and FACIT questionnaires will be administered at the visit time permitting or by phone after the completion of the in-person visit. A series of CT scans (abdomen, hip and thigh) will be done. Participants will be given their 24-hr urine collection bottle and instructions for collecting urine to measure urinary nitrogen and creatinine for estimating dietary protein intake and for storage ahead of their FV4/5 visit and asked to return it at their FV4/5 visit. Three 24-hr diet recalls will be administered by phone between during the FV4/5 visit window.

After completion of all study visits, participants will be mailed a results packet and be informed of their randomization assignment.

In the event of an abnormal laboratory value or test after randomization (e.g., eGFR), the participant will be asked to return to the clinic and provide an additional blood sample and/or have the test repeated.

Outcome Measures

The nature, purpose, and risks of all procedures and protocols will be explained to each participant before obtaining written consent. All examiners will be trained in the standardized conduct of all assessments before data collection. Participants will be instructed to wear appropriate and comfortable clothing, and standardized written instructions will be provided prior to each study visit.

Primary outcomes *(assessed at baseline and at 6-, 12- and 18-month follow-up):*

Physical performance will be assessed using a modified version of the ***Short Physical Performance Battery*** (SPPB), a widely used assessment of lower extremity physical function which includes progressively more challenging standing balance tasks held for 10 seconds each (side-by-side, tandem and semi-tandem), the faster of two 4-m walks to assess usual gait speed, and time to complete 5 repeated chair stands.[45] Each of the three performance measures is assigned a score ranging from 0 (inability to do the test) to 4 (the highest level of performance) and summed to create an SPPB summary score ranging from 0 (worst) to 12 (best). We will also administer the components of the ***expanded Short Physical Performance Battery*** (expSPPB) which was modified from the original SPPB to increase the holding time of the semi- and full-tandem stands to 30 seconds and add a single leg stand and a narrow walk test of balance (walking at usual pace within lines of tape spaced 20 cm apart). The scoring system was developed to avoid ceiling effects observed with the SPPB battery.[43] ExpSPPB scores are continuous and range from 0 to 4, with higher scores indicative of better performance.

Maximal isokinetic knee extension and flexion strength will be measured using an isokinetic dynamometer (Biodex) at two speeds (60°/sec and 120°/sec) with the participant sitting and the hips and knee flexed at 90°. The dynamometer will be adjusted for each participant and all adjustments will be recorded to duplicate the position for subsequent assessments. Start and stop angles will be set at 90° and 30°. Strength is expressed in Newton-meters (Nm). Two trials will be done consisting of 4 repetitions each. Participants who have had a brain aneurysm, cerebral hemorrhage in the last 6 months, eye surgery (e.g., cataract surgery) within the past month, blood pressure >199/109 mmHg, bilateral knee replacement, or have difficulty bending or straightening both legs due to pain, arthritis, injury or some other condition will not be tested. The maximum knee extensor strength of the 4 repetitions from trial 2 for the dominant leg will be will be used in analyses unless unable to test the dominant leg (i.e., knee replacement) in which case the non-dominant leg will be used; the maximum knee extensor strength from the same leg will be used at 6- and 18-month follow-up.

Grip strength will be measured twice in each hand using an isometric hydraulic hand dynamometer (Jamar, Bolingbrook, IL) and the mean value from the stronger hand used. Participants will be excluded from performing the test if they report hand-pain or recent hand or wrist surgery.

The ***400-meter walk*** is a walking-based test of exercise tolerance and aerobic fitness.[43] Participants are instructed to complete the 400 m distance (on a flat indoor surface) as quickly as possible at a maintainable pace and the time to complete the walk is recorded in seconds. Encouragement is given in a standardized fashion every lap. The test will be terminated if the participant is unable to continue because of pain, fatigue, or other symptom. The test has excellent reproducibility (ICC=0.95),[47] directly correlates with measured maximal aerobic fitness.[48]

Secondary outcomes *(assessed at baseline and at 6- and 18-month follow-up):*

Body composition. Fat and whole body and appendicular lean mass will be measured by dual-energy X-ray absorptiometry (DXA; Horizon A, Hologic Inc.). Whole body scans will be acquired with the participant supine and aligned with the scanner table. All scans will be performed and analyzed by our DXA technician who is certified by the International Society for Clinical Densitometry and has vast experience in body composition assessments in older adults. Daily quality control scans are obtained with Hologic's phantom. Anthropometric measures of fat distribution will be assessed by circumference measurements of the waist, hip, and thigh using a tape measure.

Thigh muscle volume and body fat distribution. Thigh muscle volume and inter-muscular adipose tissue (IMAT) and subcutaneous and visceral abdominal fat (SAT and VAT, respectively) will be measured by computed tomography (CT; Siemens SOMATOM Definition Flash 64-slice CT scanner,Siemens Medical Solutions USA, Inc., Malvern, PA). Participants will be positioned supine with the arms above the head and legs positioned flat. A 5-cm section of the thigh centered at the junction of the proximal and middle third of the femur will be used to quantify thigh muscle and adipose tissue. Thigh muscle attenuation (Hounsfield units),

excluding intermuscular fat, will also be assessed and used to quantify IMAT. CT slices within 15 mm centered at the L4-5 level will be selected to quantify SAT and VAT. Quantitative measures of thigh muscle and adipose tissue volume and CT attenuation values will be obtained with the GE Healthcare, Advantage Windows 4.2 Volume Viewer (Waukesha, WI). This software provides the ability to determine tissue-specific thresholds based on CT number (-190 to -30 Hounsfield units for fat) and volumetrically measure complex structures using semi-automated techniques such as edge attraction and volume rendering. The same CT reading technician will analyze all scans. Daily calibration scans are obtained according to the manufacturer's recommended procedure.

Tertiary outcomes *(assessed at baseline and at 6- and 18-month follow-up [Arterial Stiffness at baseline and 6-month follow-up only]):*

Bone mineral density. DXA will be used to analyze bone mineral density (BMD) using specific scans of the anterior-posterior (AP) spine (L1-L4) and proximal femur.

Volumetric bone mineral density, morphometry and strength. Helical CT scans of the lumbar spine and hip/pelvis will be used to measure volumetric bone mineral density, structure and strength of the lumbar spine (L1-L5), total hip and proximal femur. The scan coverage for the lumbar spine will be from the top of L1 through the base of L5. The hip/pelvis scan will cover the region from the superior acetabulum to mid-femur. The mid-femur position will be defined as the midpoint between the superior aspect of the greater trochanter and the inferior aspect of the lateral condyle. To locate the mid-point, an anterior-posterior scout of the entire femur will be obtained. The same Mindways bone mineral phantom (Mindways Software, Inc., Austin, TX) will be imaged in every scan.

Lipoprotein lipids. The lipoprotein lipid assays will be performed by LapCorp.

Fasting glucose and insulin. Fasting glucose and insulin assays will be performed by LapCorp. An estimate of insulin sensitivity by the homeostasis model assessment (HOMA) score will be calculated with the formula: fasting plasma insulin (μU/ml) × glucose (mmol/l)/22.5.[51] Glucose and insulin area under the curve will be determined using Tai's model: $½\times30\times(y_{0min}+2y_{30min}+2y_{60min}+2y_{90min}+y_{120min})$, where y represents insulin or glucose values at the different time points.[52]

Blood pressure will be measured in the right arm, using an automated sphygmomanometer, with the participant in a seated position after having rested quietly for 10-15 minutes. Systolic and diastolic blood pressure will be defined as the average of three repeated measures.

Arterial Stiffness measures. The SphygmoCor XCEL system is an automated device that will be used to measure central pressure waveform and corresponding indices (i.e., central blood pressure, aortic augmentation index [AIx], and reflection magnitude) using pressure oscillations from an arm cuff and a validated, generalized transfer function.[54-56] Aortic AIx is calculated as the ratio of augmentation pressure (i.e., the supplementary increase in systolic blood pressure due to early arterial wave reflections) to pulse pressure and is expressed as a percentage.[57] Reflection magnitude is determined by separating the central pressure wave into its forward and backward components and is calculated as the ratio of the forward and backward wave amplitudes.[58]

Descriptive variables and potential covariates:

Perceived fatigue and maximal aerobic capacity **(VO_2max).** Perceived fatigue will be assessed at a given workload. Participants will walk on a treadmill for 5 min at 0% grade at a fixed, comfortable speed of 2.0 mph, and heart rate and perceived exertion are recorded at midpoint and end. This test will be performed before the assessment of VO_2max and serves as a warm-up to acclimate participants to the treadmill. VO_2max will be determined on a motorized treadmill during a graded exercise test to exhaustion using a Ramp protocol to exclude those with exercise-induced ischemia at screening.[44] Dr. Nicklas and Ms. Gordon (Master's level Exercise Physiologist) have extensive experience measuring VO_2max in older persons. Throughout the treadmill test, ventilatory and gas exchange responses will be measured on a breath-by-breath basis using a computerized system (Medical Graphics), which provides valid and reliable gas analysis.[49;50] Data used to calculate the minute values will be collected for the entire 60-second period. If 60 seconds of data are not available for the last minute of the test, a minimum of 30 seconds of data must be collected and these data will be used to represent the last minute. Pre- and post-intervention testing will be conducted using the same treadmill and gas analysis system. VO_2max will be expressed both as ml of oxygen uptake per kg of body

weight per minute (ml/kg bwt/min) and per kg of lean mass per minute (ml/kg LM/min). Two of the following 3 criterion must be met for a test to be considered valid (or the test will be repeated): 1) plateau in oxygen consumption with increasing workload (< 200 ml/min); 2) respiratory exchange ratio ≥1.10; and 3) maximal HR within 90% of age-predicted maximal HR (*208 – [0.7 x age]*).

Resting metabolic rate (RMR) will be measured at baseline for prescribing calorie deficits during the weight loss intervention and at 6 months for prescribing weight maintenance diets. The test will be conducted in the morning after an overnight fast using indirect calorimetry. Upon arrival, participants are asked to rest quietly for 20-30 minutes before testing. Measurement of oxygen consumption and carbon dioxide production are collected continuously for up to 30 minutes and RMR is calculated using the Weir equation.[59]

Demographic characteristics (age, sex, race, education, and socio-economic status) will be ascertained by questionnaire at the screening visit.

A ***weight loss readiness*** questionnaire will assess participants' motivation, commitment, and any barriers to attending the 6-month weight loss intervention at the screening visit.

Medical information regarding ***co-morbidities*** will be ascertained at the screening visit. We will record ***medication and supplement use*** at baseline and at 6-, 12- and 18-month follow-up. Participants will be asked to bring in medications and dietary supplements for a "brown bag" review. The medication form will specifically ask about the use of protein supplements, anti-inflammatory agents, and sex steroids or corticosteroids.

Cognitive function will be assessed at screening using the Montreal Cognitive Assessment (MoCA),[31] participants must score ≥22 to be eligible. We will also assess psychomotor speed, attention, and working memory using the Digit Symbol Substitution Test (DSST) at baseline and at 6- and 18-mo follow-up. Participants are given a series of numbered symbols and then asked to draw the appropriate symbols below a list of random numbers. The score is the number of correctly made matches in 2 minutes (120 seconds).

Initial body weight and change in body weight: Participants will be weighed, without shoes or heavy clothing, on a standard, calibrated scale weekly during the 6-month weight loss intervention and at the baseline, 6-, 12- and 18-month follow-up visits.

Compliance: During the 6-month weight loss phase, participants will be asked to track their daily intake of all foods and beverages in a log book provided to them and their log books reviewed at each session. Attendance at group and individual diet and exercise sessions and protein supplement usage during the study will also be tracked.

Physical activity: Physical activity will be assessed by self-report at baseline, and at 6-, 12- and 18-month follow-up using the CHAMPS physical activity questionnaire.[32]

Dietary intake: Three 24-hr dietary recalls (two week days and one weekend day) will be performed on all participants at baseline, and at 6- and 18-month follow-up. Experienced registered dietitians trained in 24-hr dietary recall interviews from the WFSM Clinical Research Unit (CRU) who are not involved with the diet intervention will conduct the 24-hour dietary recalls. Data will be collected and analyzed using the Nutrition Data System for Research (NDSR) software developed and supported by the Nutrition Coordinating Center at the University of Minnesota. The NDSR database is updated annually to reflect marketplace changes and new analytic data. The program also uses the multiple pass approach, providing interview prompts to guide and standardize data entry, thereby providing multiple opportunities for the participant to recall food intake and improve data accuracy. A validated visual portion guide will be provided to the participant for use in determining accurate portion sizes. During the baseline visit, a CRU dietitian will obtain the first 24-hour recall in person, and this interview will be used to introduce and train the participant for the procedures used in subsequent 24-hr dietary recalls done by phone. The remaining 24-hr dietary recalls will be conducted over the phone (taking approximately 20-40 minutes each) by placing unscheduled telephone calls to participants. When contacted by the staff, participants will be asked to recall and report their dietary intake from the previous day's 24-hour period.

Urinary nitrogen excretion, a biomarker of dietary protein intake,[60] will be used to verify protein intake between groups. Participants will be asked to collect urine over a 24-hr period at baseline, 6- and 18-month follow-up.

Depressed mood will be assessed using the 20-item Center for Epidemiologic Study Depression Scale (CES-D)[33] at screening and at 6-, 12- and 18-mo follow-up. Individuals who score ≥16 at screening will be evaluated by the study team to determine if appropriate to participate in the study (e.g., participant did not exhibit depressed mood or a lack of interest in activities during study visit). In the event that a participant's 6-, 12- or 18-mo CES-D score increases and is above the threshold indicating that an individual is at risk for clinical depression (CES-D score ≥16) and the study team noted that the participant is exhibiting depressed mood or a loss of interest in daily activities, a letter will be sent to the participant advising them to follow-up with their PCP.

The ***Short Form-36 Health Survey (SF-36)*** will be used as a measure of quality of life and assessed at baseline and at 6-, 12- and 18-mo follow-up. The SF-36 measures 8 health domains: physical functioning, role limitations because of physical health problems, bodily pain, general health perceptions, vitality, social functioning, role limitations because of emotional problems, and general mental health.[61] Scores for each health domain subscale range from 0 to 100, with higher scores indicating better functioning or well-being.

Activities of daily living will be assessed using the Mobility Assessment Tool – Short Form (MAT-sf) at baseline, 6-, 12- and 18-month follow-up. This is a novel, computerized tool for self-assessment of functional performance designed to reduce bias from factors such as age, gender and body image.[62]

Fatigue measures. The FACIT Fatigue Scale will be administered at baseline, 6-, 12- and 18-month follow-up.

Adverse events will be assessed by asking participants at each assessment and intervention visit and on monthly phone calls during the 12-mo follow-up to report any health changes, injuries, etc., and these will be recorded using the Adverse Event Form.

Analytical Plan

Sample size: The sample size chosen for this study (n=225; 75/group) will provide ≥80% power (α=0.05) to detect clinically meaningful effects of a higher protein / lower CHO diet during weight loss and follow-up on physical function (with estimated retention of 80% or 60 completers per group; see **Table 3**). Baseline standard deviations and pre-post correlations used for power analyses were calculated using data from obese older adults from our OPTIMA study[11] – a weight loss study with the same inclusion/exclusion criteria as the proposed study. Standard deviations were assumed to be similar between groups. The mean group differences we can detect correspond to an absolute change from average baseline values of these measures in obese older adults of 0.2 points for the expSPPB and 12.6 Nm for muscle strength. Differences of 0.12 points and 0.22 points on the expSPPB have been shown to be small and large clinically meaningful differences, respectively.[63] Even with a conservative drop-out rate of 20%, 60 participants/group provides ≥80% power (overall α=0.05, 2-sided tests for 3 comparisons) to detect between group differences for primary and secondary outcomes over 18 months. In response to our NIA-appointed DSMB, we have recalculated the power based on the actual number of participants enrolled (total n=187 or ~50 per group after accounting for the 20% drop-out rate) using the same baseline standard deviations and pre-post correlations from obese older adults in our OPTIMA study noted above.

Table 3. Power to detect significant differences in mean change between groups with ANCOVA (α=0.05 after Bonferroni correction for each outcome; 2-sided test), assuming similar SD between groups

Outcome measure	Group Comparison	Pre-Post Correlation	Standard Deviation (SD)	Mean group difference	Effect Size (diff/SD)	Power for Analysis of Covariance (n per group)			
						n=60	n=65	n=75	n=50
ExpSPPB (points)	RecProt vs. 6mo HiProt	0.72	0.5	0.20	0.41	80	84	89	71
	6mo vs. 18mo HiProt			0.20	0.41	80	84	89	71
	RecProt vs. 18mo HiProt			0.41	0.82	>99	>99	>99	>99
Knee extensor strength (Nm)	RecProt vs. 6mo HiProt	0.77	32.8	12.6	0.38	81	84	90	72
	6mo vs. 18mo HiProt			12.6	0.38	81	84	90	72
	RecProt vs. 18mo HiProt			25.3	0.77	>99	>99	>99	>99
Lean mass (Kg)	RecProt vs. 6mo HiProt	0.99	12.0	1.1	0.09	86	89	93	78
	6mo vs. 18mo HiProt			1.1	0.09	86	89	93	78
	RecProt vs. 18mo HiProt			2.2	0.18	>99	>99	>99	>99

Table 3. Power to detect significant differences in mean change between groups with ANCOVA (α=0.05 after Bonferroni correction for each outcome; 2-sided test), assuming similar SD between groups

Outcome measure	Group Comparison	Pre-Post Correlation	Standard Deviation (SD)	Mean group difference	Effect Size (diff/SD)	Power for Analysis of Covariance (n per group)			
						n=60	n=65	n=75	n=50
IMAT (cm^3)	RecProt vs. 6mo HiProt	0.98	31.5	4.1	0.13	88	91	94	80
	6mo vs. 18mo HiProt			4.1	0.13	88	91	94	80
	RecProt vs. 18mo HiProt			8.2	0.26	>99	>99	>99	>99

Database management: To facilitate data transfer and preserve records that can be audited, we will use hard copy forms collection. Data will be entered into web-based forms on a continuous basis as collected and analysis datasets will be stored in SAS databases. All data will undergo range checks at the time of data entry and will be examined periodically by histograms and bivariate scatterplots to check for inconsistencies, unusual data needing further verification, and outliers. Plots of longitudinal observations will be used to inspect for unusual changes that need to be verified against source documents. Regression diagnostics and exploratory analyses will be performed to find appropriate transformations of variables if needed.

Data analysis: Initial analyses will follow the "intent-to-treat" principle in which data from all randomized participants are analyzed according to their randomized groups. Analysis of covariance (ANCOVA) will be used for both primary and secondary aims to compare the group effect on change in expSPPB and muscle strength (primary aim) and weight loss maintenance, total and appendicular lean mass and fat-to-lean mass ratio, and thigh muscle volume and inter-muscular adipose tissue (IMAT) (secondary aim) at 18 months. Group contrasts will be constructed and tested within each model. ANCOVA will also be used to compare physical function, weight and body composition at 6 months. To explore whether differences in lean mass mediate the effect of a higher protein diet during and subsequent to weight loss on physical function, change in lean mass will be added to the ANCOVA models to assess whether the intervention effect is reduced or eliminated as an ad hoc mediation analysis. To compare the exploratory outcomes (bone mineral density and cardiometabolic risk factors) at 6 and 18 months, ANCOVA with repeated measurements will be used, and correlations between 6 and 18 months taken into account using a compound symmetry covariance structure. All models will be adjusted for the baseline measure, age, gender, and race.

Factors that may influence the measured responses to the interventions (e.g., initial body weight or composition, changes in weight, physical activity, etc.) will be evaluated and possibly included in secondary analyses. We will also consider conducting analyses adjusted for intervention process measures (such as dietary intake, average attendance at diet and exercise sessions, and protein intake/urinary nitrogen) to examine the extent to which these contribute to the outcomes.

Planned sensitivity analyses to assess the impact of COVID19: Because of the potential impact of COVID19 on intervention delivery and follow-up assessments, we plan to conduct several sensitivity analyses:

1) Exclude Wave 13 and 14 participants (n=26) whose intervention was interrupted by the COVID19 research pause on March 13, 2020, and who completed the last ~2 and ~4 months, respectively, of the exercise portion of the 6-month intervention on their own while attending the diet portion of the intervention remotely (via phone).
2) Exclude Wave 13-17 participants (n=63) whose intervention was interrupted by COVID19 (Waves 13 and 14; see above) or whose intervention was modified from earlier waves to accommodate safety measures put in place due to COVID19 (Waves 15-17; center-based exercise reduced from 3x/week to 1-2x/week after week 6 to allow for physical distancing).
3) Exclude Wave 7 participants (n=15) and participants from Wave 6 (n=5) whose 18-month visits were 2-3 months out of window due to the COVID19 research pause and phased restart of in-person research.

Missing data considerations: We will document withdrawal and compliance in detail to permit the formulation of reasonable assumptions regarding missing observations. If it is determined that our outcomes are missing at random (MAR), we will attempt to identify baseline covariates that predict attrition and use these covariates to impute missing data based on multiple imputations (MI) and draw inferences based on MI. If it is determined that our outcomes are not missing at random (MNAR), sensitivity analyses MNAR assumptions will be carried out using MI following the recommendation of Molenberghs and Kenward.[64]

Human Subjects Protection

Subject Recruitment Methods: We will recruit individuals using community-based recruitment strategies including newspaper ads and mass mailings. We will also advertise in the Aging Center's VITAL newsletter and participate in community outreach events.

Informed Consent: Written informed consent will be obtained from each study participant. The informed consent process will follow the procedures of the WFU Institutional Review Board. The study interviewers will explain the purpose, methods and extent of the study to prospective participants. The potential participant is asked to read the informed consent form and ask questions. The form will be written in simple easy to understand language. We require study staff to review all of the key aspects of the study verbally with the potential participants. Staff is then required to question potential participants to ascertain whether s/he has understood the information. Potential participants who are illiterate or have impaired vision must have the consent read to them, followed by review of the checklist, opportunity for questions, and discussion. This process will take place in a quiet, private room. A copy of the signed and dated consent form will be given to participants, and the original document will be placed in subjects' individual study files, which will be stored in a secure location. In compliance with the Health Insurance Portability and Accountability Act (HIPAA) and the Standards for Privacy of Individually Identifiable Health Information of the Department of Health and Human Services, we will access personal health information only after obtaining informed consent.

Potential Study Risks: There are inherent potential risks to human subjects who participate in any research study and the potential risks to study participants in this study are listed below. Any injuries or illnesses (severe adverse events) during the course of a participant's enrollment in the study are monitored regularly as described below in the Data Safety Monitoring Plan.

1) Physical Function Testing. There is a small risk of injury during the muscle strength testing and physical performance testing, such as muscle strains or pulls, falls, or joint injury. However, these tests have been performed in large study populations with no significant adverse events reported. Risks will be minimized by having experienced/trained staff conducting these assessments. A warm-up and range of motion practice will be conducted before maximal strength testing. In addition, if a participant reports pain, dizziness, lightheadedness or other medical problem during the test, the test will be terminated.

2) Radiation Exposure. There are risks with exposure to radiation from the whole body, anterior-posterior spine, and proximal femur DXA scans and chest, abdominal, pelvic and thigh CT scans. Exposure to radiation during the DXA (1-10 mRem each) and CT (137-255 mRem each) scans for a total of 1860 mRem of radiation exposure from the DXA and CT scans over the three time points during the 18 months of the study which is lower than the maximal permissible dose for occupationally exposed individuals. The potential long-term risk from these radiation doses is uncertain, but these doses have never been associated with any definite adverse effects. Thus, the risk to participants, if any, is estimated to be slight.

3) Blood Draw. Participants may experience temporary pain, bruising, bleeding and/or a small risk of infection, fainting or dizziness during the blood sample collection process. Blood will be drawn only by trained and experienced phlebotomists who will minimize the discomfort as much as possible and will use good clinical practice procedures to reduce risk of infection.

4) Exercise testing is a common procedure with minimal risks, but the test is monitored by an exercise physiologist and will be stopped if problems occur. These include fainting, dizziness, chest pain, irregular heartbeats, or a heart attack, although the latter is extremely rare (<1 death in 10,000 tests) in people with no history of heart disease. Blood pressure, heart rate and rhythm, and breathing will be closely and constantly monitored.

5) Resting Metabolic Rate. Some participants begin to feel claustrophobic when the clear plastic mask is placed over their mouth. Should this occur we will remove the mask and stop the test.

6) Risks of weight loss. There are no known serious risks associated with using caloric restriction to lose weight. Changes in usual bowel function (diarrhea and/or constipation) may occur when beginning the new diet due to differences between the weight loss diet and the participant's usual diet. Under conditions of rapid weight loss (more than 5 pounds per week), there is a very small chance of developing gallbladder disease. Risks at any age include the concomitant loss of lean (muscle and bone)

tissue along with fat mass loss. However, the clinical impact of this muscle and bone loss is not known. In older adults, this decrease in muscle mass and bone density with weight loss may result in increased risk for sarcopenia and/or osteopenia/osteoporosis. Since most older adults with obesity have a higher muscle mass and bone density, this risk is mitigated by excluding individuals who are non-obese and less likely to benefit from weight loss and excluding those with an increased risk for sarcopenia or osteoporosis. Individuals with T-score $\leq$ -2.5 on hip or spine DXA scan at screening will be excluded. Procedures to minimize loss of muscle and bone during the weight loss intervention include prescription of a caloric deficit that does not result in excessively rapid weight loss (e.g., >2 lb/week), inclusion of increased weight-bearing activity (exercise intervention), and incorporating dietary recommendations and meal plans that include the current recommended dietary allowance (RDA) for protein (0.8 g/kg body weight/d) and counseling to consume, either from food or dietary supplements, 1200 mg/d of calcium and 800 IU/d of vitamin D.

7) The risks of the higher protein diet intervention are minimal, but may include a worsening of existing kidney problems. However, we will not enroll individuals with evidence of preexisting impaired kidney function for whom a diet with a higher protein level is contraindicated (i.e., eGFR<30 based on serum creatinine, age, gender, and race).

8) Exercise Program. The risks of the exercise program are minimal, but may include musculoskeletal complications and muscle soreness in the early phases of the training. This will be minimized since all exercise sessions during the first 6 weeks of the 6-month weight loss intervention and up to 2 sessions per week for the remainder of the 6-month weight loss intervention will be center-based and supervised by trained exercise interventionists, who will instruct participants in the proper exercise techniques and proper footwear (unless conditions arise that necessitate a transition to all home-based exercise – e.g., increased community spread of COVID19, new restrictions on in-person research are put in place, and/or if stay at home orders are put back in place). Procedures to minimize injury include warm-up and cool-down activities that include large muscle movements and stretching. Participants will begin with an easier exercise stimulus and will gradually increase in intensity over several weeks. Exercise physiologists, trained in cardiac life support, will supervise all center-based exercise sessions and practice codes are conducted quarterly. Tips on how to safely exercise in a home-based setting will be reviewed by the exercise interventionist during the first 6 weeks of center-based exercise.

Confidentiality and Privacy

All data are obtained for research purposes only. Confidentiality of data is maintained by using research identification numbers which uniquely identify each individual. Data will be used only in aggregate and no identifying characteristics of individuals will be published or presented. Results of testing will be sent to participants' private physicians if participants agree to this. Confidentiality of data is maintained by using research identification numbers which uniquely identify each individual. The information collected from participants in this study has a low potential for abuse because the data does not address sensitive issues. Nevertheless, appropriate measures are taken to prevent unauthorized use of study information. Data other than demographic information do not use names as an identifier – the research ID number is used. The research records are kept in locked cabinets in the Geriatric Research Clinic. The files matching the participants' names and demographic information with the research ID numbers are kept in a separate room and locked file that uses a different key from that of all other files. A web-based data entry system will be created for this study which will allow access only to study staff (user id and password required to access). Information linking IDs to individuals is kept on a secure, password-protected server to which only authorized study personnel will have access. Blinded staff will not have access to blinded study information on the web-based database. Computer files are stored on file servers, which are backed up each night and stored for easy retrieval in case of emergency. Files may not be obtained from the research unit by persons other than research personnel, who are asked to sign a document agreeing to maintain the confidentiality of the information. After the study is completed, the local data will be stored with other completed research studies in a secured storage area.

Data and Safety Monitoring

The PI, along with the study physician, will be responsible for the overall monitoring of the data and safety of study participants. In addition, all clinical intervention studies conducted by Aging Center investigators are monitored by the WFU Claude D. Pepper Older Americans Independence Center's NIH-approved Data Safety

Monitoring Board (DSMB). Finally, the external NIA-appointed Data and Safety Monitoring Board (DSMB) will have overall responsibility of independently monitoring all aspects of the study, including those that require access to any un-blinded data. This committee acts in an advisory capacity to the NIA to monitor participant safety, evaluate the progress of the study, review procedures for maintaining the confidentiality of data, and the quality of data collection, management, and analyses. The DSMB will have access to all study data, documents and progress information, and will be notified of all changes that are made to the protocol.

The principal investigator will be responsible for the overall monitoring of the data and safety of study participants. The principal investigator will be assisted by other members of the study staff. The PI, co-investigators, and the biostatistician will meet regularly to review study progress and to examine reports of adverse incidents as well as participant recruitment and follow-up. All *serious* adverse events will be reported to the Institutional Review Board (IRB) and if the event occurred as a direct result of participation in this study, an amendment will be made to the consent form and the PI will request new IRB approval. The PI and the study physician (Dr. Lyles) will meet quarterly, or as needed, to review all reported events. In addition, participant safety in all Wake Forest Pepper Center supported studies is monitored by a Data and Safety Monitoring Committee (DSMC). It reviews study recruitment, drop-outs, protocol changes, losses to follow-up, and adverse events every 6 months.

Adverse events include any event that occurs during the course of the study that results in a participant suffering physical or mental injury, pain or suffering. Adverse events can be major, such as a subject who suffers cardiac arrest during neuromuscular function testing, or minor such as a subject pulling a muscle during neuromuscular function testing. This includes any events occurring while a subject is enrolled in the study, even if the event did not occur while s/he was actively participating in the activities called for in the research protocol. Deviations from the study's protocol are also considered an adverse, unexpected, or notable event and will be reported to the PI.

Both major and minor events will be reported using the study's Adverse, Unexpected, or Notable Event Reporting Form. Any major related and unexpected event, i.e., any serious injury or life-threatening event, will be reported immediately (within 24 hours of notification) to the WFSM Institutional Review Board (IRB) right after completing any and all actions that are necessary to protect the subject's health and safety. The SAE will also be reported within 24 hours to the NIA and Chair of the DSMB via a verbal phone call, or by a report submitted by fax or email. Minor events will be reported within seven days. A description of the event, and the date and location of the event will be recorded on this form, which will be kept in the subject's research file.

Reporting of Unanticipated Problems, Adverse Events or Deviations

Any unanticipated problems, serious related and unexpected adverse events, deviations or protocol changes will be promptly reported by the principal investigator or designated member of the research team to the IRB and sponsor or appropriate government agency if appropriate.

Use of biological samples by other investigators

Biological samples may be used by investigators other than the investigators of the current study. The use will be limited to non-commercial purposes. The names and other personal identifiers of the study participants will not be sent to any recipients of the blood samples.

Storage and disposal of biological material

Blood and urine samples will be stored at Wake Forest University Medical Center for up to twenty years after the end of the trial at which time the samples will be destroyed. Biological specimens will be stored in locked -70^{o}C alarmed freezers located in a locked room. The Wake Forest Pepper Center Integrative Biology Core lab coordinator (Karin Murphy) and the Core Leader (Dr. Nicklas) have access to the keys of the freezers. All the specimens will have numerical study IDs with no personal identifiers of the participants. These are stored under the Pepper Center Tissue Repository (IRB#1219).

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