The Vitality, Independence, and Vigor in the Elderly 2 Study (VIVE2): Design and Methods

Dylan R. Kirn¹, Afsaneh Koochek², Kieran F. Reid¹, Åsa von Berens², Thomas G. Travison^{6,7,13}, Sara Folta⁴, Jennifer Sacheck⁴, Miriam Nelson^{4,5}, Christine Liu^{1,9}, Edward Phillips^{1,7,8}, Anna Cristina Åberg^{10,11}, Margaretha Nydahl¹², Thomas Gustafsson³, Tommy Cederholm², Roger A. Fielding¹

1 Nutrition, Exercise Physiology, and Sarcopenia Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts.

2 Clinical Nutrition and Metabolism, Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden.

3 Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden.

4 Friedman School of Nutrition Science and Policy, Tufts University, Boston, Massachusetts.

5 Jonathan M. Tisch College of Citizenship and Public Service, Tufts University, Medford, Massachusetts.

6 Institute for Aging Research, Hebrew SeniorLife, Boston, Massachusetts.

7 Harvard Medical School, Boston, Massachusetts.

8 Spaulding Rehabilitation Hospital, Boston, Massachusetts.

9 Section of Geriatrics, Boston University School of Medicine, Boston, Massachusetts.

10 Geriatrics, Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden

11 School of Education, Health and Society, Dalarna University, Sweden

12 Department of Food, Nutrition and Dietetics, Uppsala University, Uppsala, Sweden.

13 Division of Gerontology, Beth Israel Deaconess Medical Center, Boston, Massachusetts

Address correspondence to Dylan R. Kirn, Nutrition, Exercise Physiology, and Sarcopenia Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington Street, Boston, MA. Phone: (617) 556-3399. Email: Dylan.kirn@tufts.edu

Word Count (inclusive of tables): 5,497

Abstract

Background: Nutritional supplementation may potentiate the increase in skeletal muscle protein synthesis following exercise in healthy older individuals. Whether exercise and nutrition act synergistically to produce sustained changes in physical functioning and body composition has not been well studied, particularly in mobility-limited older adults.

Methods: The VIVE2 study was a multi-center, randomized controlled trial, conducted in the United States and Sweden. This study was designed to compare the effects of a 6-month intervention with a once daily, experimental, 4 fl. oz. liquid nutritional supplement providing 150 kcal, whey protein (20 g), vitamin D (800 IU) (Nestlé Health Science, Vevey, Switzerland), to a low calorie placebo drink (30 kcal, non-nutritive; identical format) when combined with group-based exercise in 150 community-dwelling, mobility-limited older adults. All participants participated in a structured exercise program (3 sessions/week for 6 months), which included aerobic, strength, flexibility, and balance exercises.

Results: The primary outcome was 6-month change in 400 m walk performance (m/s) between supplement and placebo groups. Secondary outcomes included 6 month change in: body composition, muscle cross-sectional area, leg strength, grip strength, stair climb time, quality of life, physical performance, mood/depressive symptoms and nutritional status. These outcomes were selected based on their applicability to the health and well-being of older adults.

Conclusions: The results of this study will further define the role of nutritional supplementation on physical functioning and restoration of skeletal muscle mass in older adults. Additionally, these results will help refine the current physical activity and nutritional recommendations for mobility-limited older adults.

Key words: older adults, mobility-limitations, physical activity, protein, vitamin D, supplementation

Introduction

Mobility limitations, such as the inability to rise from a chair or ascend a flight of stairs, affect many older adults. Mobility-limited older adults experience higher rates of falls, institutionalization, and chronic disease.[1] As the worldwide population of persons 70 years and older continues to increase in the United States[2] as well as in Europe,[3] there is a substantial need for interventions to address the inevitable increase in mobility limitations among this population.

Inadequate physical activity and low protein intake are two factors that contribute to the decline in muscle mass and function associated with aging (sarcopenia).[4-6] Though several studies have shown a positive impact of physical activity in this population, [7-9] studies investigating protein supplementation in combination with exercise have been mixed.[10-17] Several lines of evidence have suggested that nutritional supplementation (particularly supplements high in protein) can potentiate the increase in skeletal muscle protein synthesis following a single bout of exercise in both young individuals and in healthy older adults,[18-21] though the effects in mobility-limited older adults remains unclear. More recently, poor vitamin D status has emerged as a potential contributor to poor muscle function among older adults.[22] Observational evidence suggests that individuals with deficient or insufficient vitamin D levels are more functionally limited compared to those who are sufficient.[23] Additionally, cross-sectional studies have shown that vitamin D status is highly correlated with lower-extremity strength and balance.[24-27] Though it has been hypothesized that, vitamin D supplementation with and without exercise may decrease incidence of falls and fractures in the elderly, the results of these trials have been mixed.[27-29] However, both cross-sectional and randomized controlled trials with vitamin D supplementation that investigate muscle function have had conflicting results, and therefore this relationship requires further investigation.[30, 31]

To date, no studies have been conducted to determine whether synergistic effects of exercise and nutrition can result in sustained increases in physical functioning and fat free mass. Such investigations would be of particular relevance to older adults with demonstrated functional limitations, who are at

disproportionate risk for mobility disability. The Vitality, Independence, and Vigor 2 Study (VIVE2) described here was designed to compare the effects of an intervention with an experimental liquid oral nutritional supplement providing 150 kcal, high amounts of whey protein (20g), high amounts of vitamin D (800 IU) in a small volume (4 fl. oz., ca. 119 ml; (Nestlé Health Science, Vevey, Switzerland), to a low calorie placebo drink (ca. 30 kcal, no protein, no Vitamin D; identical format) on exercise training-induced changes in physical functioning in older adults with demonstrated mobility limitations and vitamin D insufficiency (≤ 60 nmol/l) across two international field centers.

Primary Research Outcome

The primary outcome of the VIVE2 study was 6 months change in walking speed, as derived from the 400 Meter Walk test [32]. The design was intended to facilitate comparison of the effects of a 6 months structured physical activity program in combination with daily high protein, high vitamin D nutritional supplementation or placebo on changes in walking speed and other outcomes, using data on 150 older adults with limitations in mobility at baseline.

Methods

Overview of Study Design

This study was designed as a double-blind, placebo-controlled, multicenter, randomized controlled trial. Subjects were enrolled at two field centers: Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University in Boston, MA (BOS) and Uppsala University/Karolinska Institutet in Stockholm, Sweden (SWE). These sites were chosen, as both countries have a rapidly growing population of older adults, which in-turn leads to an increase in the prevalence of mobility-limitations and vitamin D insufficiency. This study was approved by the Tufts Health Sciences Campus Institutional Review Board, as well as the Regional Ethical Committee of Uppsala. All enrolled participants participated in a moderate intensity exercise program, and were randomized to receive a high

protein, high vitamin D nutritional supplement or a placebo. The exercise and supplement/placebo intervention was 6 months in duration. Outcomes were assessed at baseline, 3, and 6 months.

Eligibility

The VIVE2 eligibility criteria can be found in Table 1.

Table 1: Inclusion/Exclusion Criteria

Inclusion Criteria
Males and Females age ≥ 70 years
Community dwelling
Short Physical Performance Battery ≤ 9
Willingness to be randomized and come to the laboratory for 6 months
Body Mass Index ≤ 35
Mini mental state examination ≥ 24
Serum 25 (OH) D 22.5-60 nmol/l
Having obtained his/her informed consent
Able to complete 400 M walk within 15 minutes
Exclusion Criteria
Current Health Status
Acute or terminal illness
Myocardial infarction in previous 6 months, symptomatic coronary artery disease, or congestive heart failure
Upper or lower extremity fracture in previous 6 months
Uncontrolled hypertension ($>150/90$ mmHg)
Hemoglobin < 10 g/dL
Estimated GFR < 30 mL/min/1.73m ²
Major surgery in the past 6 months (requiring general anesthesia)
Other significant co-morbid disease that would impair ability to participate in an exercise-based intervention, e.g. renal failure on hemodialysis, severe psychiatric disorder (e.g. bipolar, schizophrenia)
Wheelchair bound
Inability to communicate due to severe, uncorrectable hearing loss or speech disorder
Severe visual impairment (if it precludes completion of assessments and/or intervention)
Severe progressive, degenerative neurologic disease
Severe rheumatologic or orthopedic diseases, e.g., awaiting joint replacement, active inflammatory disease
Terminal illness with life expectancy less than 12 months, as determined by a physician
Cancer requiring treatment in the past 3 years, except for non-melanoma skin cancers or cancers that have clearly been cured or in the opinion of the investigator carry an excellent prognosis (e.g., Stage 1 cervical cancer)
Severe pulmonary disease, requiring either steroid pills or injections or the use of supplemental oxygen
Severe cardiac disease, clinically significant aortic stenosis, history of cardiac arrest, use of a cardiac defibrillator, or uncontrolled angina
Nutritional Exclusions
Current regular use of high protein oral supplements (> 1 per week)
Vitamin D supplements >800 IU/day
Milk protein allergy
Excessive alcohol use (>14 drinks per week)
Physical Activity
Participation in moderate intensity activity >20 minutes/week
Behavioral
Patient who cannot be expected to comply with treatment as decided by the Principal Investigator
Conditions not specifically mentioned above may serve as criteria for exclusion at the discretion of the clinical site Principal Investigator and/or study Physician

Recruitment

A total of 150 subjects were planned to be recruited across both study sites (80 subjects in BOS, and 70 in SWE).

Recruitment Strategies

The recruitment at BOS included a direct mailing campaign of approximately 400,000 letters and brochures, community-outreach strategies at local senior centers and senior living facilities, and local newspaper advertisements. At SWE, the primary recruitment strategy was repeated advertisements in a local paper distributed for free in the southern part of Stockholm County. Approximately 400 letters were also mailed out to older adults living in the southern suburbs of Stockholm. Moreover, posters and brochures were distributed to local senior centers and primary health care centers in the southern part of Stockholm County.

Screening

Potential participants were initially interviewed over the telephone to assess eligibility. Those who qualify were asked to attend an orientation screening visit (OSV) at the site.

The screening process was divided into two separate visits. OSV sessions were held 2 to 3 times per week. The OSV was conducted in a group format, where approximately 7-15 participants would attend each session. A brief presentation discussing the study background and requirements was provided by a study coordinator. Those who decided to participate performed the SPPB with study staff. If participants were eligible following the SPPB (score ≤ 9), a small blood sample would be obtained to measure serum 25 (OH) D level (DiaSorin RIA kit (DiaSorin, Stillwater, MN, USA)). Subjects screened at SWE performed the 400 M walk at the OSV in addition to the SPPB screening and blood sample. The 400 M walk was added to the OSV at SWE to address logistical obstacles to the local screening process, and it was agreed by the investigators across both sites that this would not introduce any additional bias

into the selection of study participants, as the same inclusion criteria was utilized across both sites.

Subjects who are eligible after the OSV were invited back to the site for evaluation of the remaining eligibility criteria. Subjects found to be eligible for randomization signed the study informed consent form prior to randomization. As indicated in exclusion criteria in Table 1, the site PI can exclude a participant if it is believed that they may not be appropriate for a group-based intervention. In our previous work, participants who fall into this category are typically excluded for behavioral reasons, which may have influenced the adherence of other participants, given the group-based design of the study intervention. If one site was unsure about the inclusion of a participant, it would be discussed with the study team across both sites. Though the use of this exclusion criterion is rare, the investigators agreed that this is important to include in a study of this design.

Informed Consent

Informed consent was collected prior to each screening visit, as a separate screening informed consent was utilized to maximize the efficiency of these visits. Participants were provided a copy of the informed consent to read at home prior to each visit, and were provided ample time to review the study details with the study staff prior to signing. In addition, a separate tissue banking informed consent was used to obtain consent for retaining biological samples for future unspecified use.

Study Intervention

Physical Activity

All randomized subjects were asked to participate in an exercise program designed by study investigators in collaboration with Nestlé Health Science, Vevey, Switzerland. Participants began the intervention on a rolling basis, and class size ranged from 1 to 15 participants depending on the study stage (the first and last participants enrolled had a brief period of exercising alone, up to the peak where up to 15 participants would attend a class). Class sizes were not to exceed 15 participants in order to ensure safety throughout each session. At least two trained and certified interventionists supervised each

class. Participants were asked to attend three center-based exercise sessions per week, which were conducted in a supervised setting, similar to previous interventions in this population.[9] These sessions were used to initiate the aerobic program and to introduce participants to the strength, stretching, and balance portions of the program in a safe and effective manner. The supervised setting allowed instructors to better tailor the program to individual needs and abilities early on, so as to prevent early dropout and to facilitate the building of self-efficacy and support, which have been found to be key to long-term physical activity maintenance.[37, 38] These exercise sessions involved approximately 60 minutes of exercise instruction including 30 minutes aerobic, 20 minutes strength (using ankle weights), a warm-up, and cool down. The strength exercises included chair rises, knee extension, side hip raises, knee flexion, and calf raises. The warm-up and cool down portions include flexibility and balance exercises. Rest periods were built in between the varying types of exercise throughout each session (aerobic and strength, strength and balance).

The participants were introduced to the activities of the physical activity intervention in a structured way such that they begin with lighter intensity and gradually increase intensity over the first 2-3 weeks of the intervention. Given the limited mobility of the participants at baseline, many had to work up to completing the full 60 minutes of exercise throughout the intervention. VIVE2 promoted walking for exercise at a moderate intensity and relied on ratings of perceived exertion as a method to regulate physical activity intensity. Using Borg's scale,[39] that ranges from 6 to 20, participants were asked to walk at an intensity of 13 (activity perception SOMEWHAT HARD). They were discouraged from exercising at levels that approach or exceed 15 (HARD) or drop to a rating of 11 (FAIRLY LIGHT) or below. As participants continued to participate throughout the trial, the total amount of minutes walked at each session was gradually increased by the study interventionists. Lower extremity strengthening exercises were performed (2 sets of 10 repetitions) at an intensity of 15 to 16 using Borg's scale. The strength exercises also gradually increased in intensity throughout the intervention period. The balance exercises were also introduced gradually. The battery of balance exercises were categorized into 5 levels,

where level 1 was the easiest, and level 5 was most difficult. Participants were introduced to the balance exercises at level 1, and were progressed to the more difficult levels throughout the course of the intervention.

The subjects were also encouraged to conduct physical activity outside of class as well. The overall goal of the physical activity program was for participants to complete at least 150 minutes per week of moderate intensity physical activity. This is consistent with current public health guidelines for physical activity by older adults.[40] Subjects were instructed to record any physical activity performed (apart from the exercise classes) on a home log provided by the study interventionists. All participants were instructed not to commence any new exercise or rehabilitation program during the study, but are not instructed on levels of voluntary or spontaneous activity.

Nutritional Supplement

The experimental study product was a ready-to-drink, liquid nutritional supplement in a small volume (4 fl. oz. or ca. 119 ml). The supplement was delivered to the sites directly from the study sponsor (Nestlé Health Science, Vevey, Switzerland) in a prepared, liquid form. At SWE, the nutritional supplement was sent monthly, directly to study participant's homes. It was nutrient-dense, providing 150 kcal/serving, and provided whey protein in a proprietary concentrated form (20g/4 fl. oz.), the daily recommended intake of vitamin D (800 IU) for older adults [41], together with a whole set of macro-and micronutrients. The placebo was a low energy flavored drink (ca. 30 kcal), not containing protein and vitamin D.

Each participant was instructed to consume one serving (4 fl. oz.) per day. The participants were either consuming the supplement or the placebo. On exercise days, the participants would drink one beverage immediately following the exercise class, before leaving the site. This procedure was put in place as it can impact adherence and reduce the likelihood that the participant may forget to take it. It also provides a venue for participants to discuss any concerns they may have with the study product.

Adherence and Retention Strategies

Due to the high number of co-morbidities observed with this population, it was very important to have a plan in place to maximize retention rates. For this study, we assumed an attrition rate of approximately 20%, as this is consistent with other clinical trials in this population. At BOS, transportation was offered to study volunteers, as many older volunteers have difficulty commuting into the urban-based center, and many volunteers resided in the suburban areas of Boston. Similarly, transportation programs at SWE that are publicly available were advertised and were utilized as needed.

In addition, this study was designed as a group-based exercise intervention to promote socialization, and to create a group-like atmosphere, which would assist in keeping participants, engaged and motivated to attend. In addition, several classes were offered throughout the week in order to provide a flexible schedule. Though participants were encouraged to adhere to a regular schedule, offering classes throughout the week allowed some flexibility should outside commitments arise.

In order to maximize adherence to the nutritional supplement/placebo, participants were asked to record whether or not they consumed their beverage each day on a study log. In addition, there was a space to record any unintended adverse effects that may be due to the study product.

Risk

As this study was performed in a mobility-limited population, unique measures were required to reduce risk. Though the risk of participating in moderate-intensity physical activity was low, some risks remain, such as muscle soreness, and falls. However, this study employed a highly rigorous screening process, in order to address certain individual's eligibility and safety for completing the exercise program. In addition, all exercise classes were supervised by trained and certified study staff interventionists, who thoroughly instructed participants on correct form, appropriate level of exertion, and were able to assess safety during the study intervention. The intervention staff members would adjust the intensity of the

exercise performed by each individual subject, to ensure that the exercises were performed effectively and safely.

Though the risks associated with the nutritional supplement were low, it was considered very important to collect any information on any possible adverse effects. Participants were asked to record any adverse effects they experienced that may have been due to the study product on their weekly logs, and these were reviewed regularly by the study staff. If participants reported any adverse effects that they believed to be attributed to the study product, the study physician may decide to reduce the dose or temporarily withhold the study product to see if the side effects subside. Pending the outcome, appropriate action would be taken by the study team.

Study Measures

In order to assess the efficacy of the supplement combined with exercise, all outcomes were assessed at baseline and 6-months. Moreover, in order to examine the trajectory of change among some of the functional outcomes, a shortened assessment was performed after 3 months of the intervention. In addition, as there is a long list of outcomes for this study, the baseline visit was separated into two visits, each approximately 2-3 hours in length, in order to avoid fatigue. This process was repeated at 6-months. The full schedule of assessments can be found in Table 2.

Table 2: Schedule of Assessments

Assessments	Screening	Baseline	3-Month Visit	6-Month Visit
SPPB	X		X	X
MMSE	X			
ECG	X			
Height	X			
Weight	X		X	X
Medications	X		X	X
Vital Signs	X		X	X
History and Physical Examination	X			
400 M Walk	X		X	X
Peak Torque and Power		X		X
Questionnaires		X	X	X
Stair Climb Test		X		X
Grip Strength Test		X		X
Accelerometry		X		X
Computed tomography		X		X
DXA Scan		X		X
Muscle biopsy		X		X

Primary Study Outcome

The primary study outcome was a comparison of change in 400 M walk performance between nutritional supplementation and placebo (expressed as average gait speed during the 400 M walk (m/s)), after 6 months of moderate intensity exercise. Change in 400 M walk time at 3 months was considered a secondary outcome.

The 400 m walk was conducted in a long, straight hallway, where two cones mark a 20 meter course. Participants were instructed to walk 10 laps around the cones at their usual pace, without over-exerting themselves. Participants must have been able to complete all 10 laps in less than 15 minutes in order to be enrolled in the trial. A straight cane may have been used, and participants were allowed to stop to rest, though leaning on any other object or sitting was not allowed during the test.[42, 43]

Secondary Study Outcomes

Stair climb: Change in stair climb time from baseline to 6 months was considered a secondary outcome. Stair climb time was assessed using a standard set of stairs as described previously.[44] The subject was asked to ascend a set of 10 stairs as quickly as possible. Two trials were conducted with 60 seconds of rest separating each trial. Subjects could ascend holding the railing or using their assistive device if necessary. Time was recorded using the Lafayette Instruments Multi-function Timer/Counter (Lafayette Instruments, Lafayette, IN). The average of the 2 attempts was recorded.

SPPB: Change in SPPB score from baseline to 6 months was considered a secondary outcome. The SPPB captures domains of strength, endurance, and balance and is highly predictive of subsequent disability.[33] The test consists of 1.) a simple test of balance, involving three different foot positions, that are required to be held for 10 seconds, 2) four meter gait speed, and 3) a repeated chair rise. Each component is scored using a 0-4 scale and the composite score is reported from 0-12.

Dual Energy X-Ray Absorptiometry (DXA): Total body lean mass and composition was measured using DXA (Hologic, Discovery A (Hologic, Bedford MA) used at BOS; GE Lunar (Madison, WI) used at SWE).[45] Change in total body lean mass was considered a secondary outcome. The DXA system generates photons at two principal energy levels (40 and 70 KeV) which allow measurement of bone mineral and soft tissue. Whole body and regional fat and fat free mass were assessed for this study.

Computed Tomography (CT): Regional changes in mid-thigh skeletal muscle cross sectional area were assessed by CT. Scans of the non-dominant thigh were performed at the midpoint of the femur for each subject. The length of the femur was determined from a coronal scout image as the distance between the intercondylar notch and the trochanteric notch. All scans were obtained using a Siemens Somatom Scanner (Erlangen, Germany) operating at 120 KV and 100 mA. Technical factors included a slice width of 10 mm and a scanning time of 1s. All scans were analyzed by a single investigator in a blinded manner using SliceOmatic v4.2 software (Montreal, Canada). Images were analyzed for muscle cross sectional

area, and subcutaneous and intermuscular fat cross sectional area. This method of quantifying mid-thigh muscle cross sectional area has been used in many previous trials.[46-50]

Isokinetic Peak Torque and Isometric Strength: Isokinetic peak torque and isometric strength was determined for the knee flexors and extensors using the Biodex System 3 Isokinetic Dynamometer (Biodex Medical Systems, Shirley, NY). Peak torque measurements were obtained with the subjects seated and the knee fully extended. Peak torque was measured at 60, 90, 180 and 240°sec⁻¹. These specific angular velocities were selected because of their association with functional performance and good reliability in this population.[51] For the measurement of isometric strength, participants alternated between extension and flexion repetitions (30 seconds rest between each repetition). Participants were instructed to apply as much force as possible for 5 seconds. Isometric strength for knee extensors and flexors was considered the peak force generated across three isometric repetitions for each leg.

Hand Grip Strength: Hand grip strength was measured in the dominant hand with a Jamar Handheld Dynamometer (Patterson Medical, Warrenville, IL).[52] The patients performed the test while sitting comfortably with shoulder adducted and neutrally rotated, the elbow supported on a table and flexed to 90 degrees, and the forearm and wrist in neutral position. The patients were instructed to perform a maximal isometric contraction. The test was repeated after 10 seconds and the highest value is recorded.

Mini Nutrition Assessment Short Form, (MNA® -SF): The MNA® -SF is a validated nutrition screening and assessment tool that can identify geriatric patients age 65 and above who are malnourished or at risk of malnutrition.[53] Change in nutritional status based on the MNA-SF was considered a secondary outcome.

Mood/depressive symptoms: Mood and Depressive Symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D). The CES-D scale is a short self-report scale designed to measure depressive symptomatology in the general population.[54]

Quality of life: Quality of life was assessed by employing the validated Medical Outcomes Study 36-item Short-Form General Health Survey (SF-36).[55] The questionnaire consists of 36 questions, is self-administered and assesses quality of life and wellbeing in eight multi-item scales regarding physical functioning and perception of physical role, vitality, general and mental health, perception of emotional role, social functioning and bodily pain.

Exploratory Outcomes

Accelerometry: To evaluate the influence of both the nutritional supplement and the physical activity intervention on changes in spontaneous/voluntary physical activity, accelerometry was performed using the Actigraph Model GT3X+ (Actigraph, Pensacola, FL).[56] The accelerometer was attached to an elastic belt which wraps around the subject's waist. The accelerometer was positioned superior to the iliac crest. Participants were instructed to wear the accelerometer for a 7-day period during waking hours and remove it for sleep and bathing only. Both the volume and intensity of physical activity was quantified.

Insulin Sensitivity: A 120-minute oral glucose tolerance test (OGTT) was performed to assess responses in insulin sensitivity to the study intervention. Measurements of plasma glucose, insulin and c-peptide were obtained at 30 minute intervals (0, 30, 60, 90, and 120 minutes). Area under the curve (AUC) analysis was used to quantify changes in insulin sensitivity between the two treatment groups.[57]

Muscle samples: Muscle biopsies were obtained from a subset of study participants to investigate vitamin D molecular pathway activation when subjects perform physical activity and consume a nutritional supplement or placebo.

Compliance: Compliance with the physical activity intervention was assessed by attendance at the scheduled physical activity sessions. A compliance log was also given to document physical activity done in between physical activity sessions. Compliance with the nutritional supplements was monitored using a compliance log that each subject was instructed to complete.

Biological samples: Blood samples were collected to examine standard laboratory analytes, blood lipids and hematology, acute phase proteins, and circulating cytokines. A detailed analysis plan and collection schedule can be found in Table 3.

Table 3: Biological Sample Analysis Schedule				
Time point	Screening	Baseline	3-Month Visit	6-Month Visit
BLOOD				
Serum 25 (OH) D	X	X	X	X
Standard Laboratory Analytes, blood lipids, and hematology	X		X	X
Acute Phase Proteins <i>hsCRP</i> <i>Albumin</i> <i>Fibrinogen</i>		X	X	X
Cytokines <i>IL-6</i> <i>TNF-α</i>		X	X	X
Growth Factors <i>IGF-1</i>		X	X	X
OGTT <i>Insulin</i> <i>C-peptide</i> <i>Plasma Glucose</i>		X	X	X
URINE				
Urinalysis	X			

Planned Statistical Considerations

The planned sample size (N=150) will provide 90% power to detect a clinically significant difference of 0.1 m/s in average walking speed over 400 m assuming walking speed has a standard deviation of 0.19 m/s, Pearson correlation between baseline and follow-up measurements at least 0.5, and cumulative missingness of walking speed measurements no greater than 20% at 6 months.

The primary target of estimation is the mean difference in walking speed (m/s), derived from the 400m walk test, attributable to nutritional supplementation after 6 months of moderate intensity exercise. This will be estimated using a mixed-effects linear regression of walking speed on intervention (vs. placebo) with control for sex (a stratification factor) and baseline walking speed, with study center (Boston or Sweden) included as a random effect. Treatment effects will be estimated using treatment contrasts and associated 95% confidence interval estimates. A secondary analysis will utilize mixed effects regression models of 3 and 6 month changes in walking speed simultaneously, with participants clustered within study centers. Analysis of continuous secondary endpoints will be conducted using the same approach as described for the primary endpoint. Analysis of discrete and time-to-event outcomes will be developed using relevant approaches; for instance, between-group differences in incidence of fall will be assessed using the Kaplan-Meier method and proportional hazards regression models. Both intention-to-treat and completer analysis will be utilized for this study data. The completer analysis will include all participants who attended at least 60% of the exercise sessions and utilized at least 60% of the planned supplementation.

Blinding

Strict measures were in place to ensure study blinding to this study. All study staff, including staff conducting the intervention, outcomes, as well as the principal investigators were all blinded to treatment group (supplement vs. placebo). Randomization was performed electronically, and could be broken immediately if an emergency situation arose.

Randomization

Randomization of subjects to the nutritional supplement or placebo groups was designed to stratify for gender, and utilize a separate blocked randomization schedule for males and females. Based on our laboratory's prior experience, it was anticipated that our sample will be comprised of a 2:1 ratio of female to male subjects. A block size of 10 was employed (allocating 5 subjects to the supplement group

and 5 subjects to the placebo group within each sex). A computer-generated randomization determined the order in which the supplements are assigned in each block. The group assignments based on this randomization scheme were administered by a research assistant not affiliated with this study and who had no direct contact with the research participants, or study staff.

Discussion

The primary aim of this study was to compare the effects of a high protein, high vitamin D nutritional supplement with a placebo on exercise-induced changes in physical functioning older adults. Multiple studies have examined the impact of protein supplementation on strength, muscle size, and muscle quality when combined with resistance training in older adults.[13, 36, 58-60] However, this study was the first to examine the long term effects of a low volume ready-to drink liquid nutritional supplement providing whey protein in a proprietary concentrated form (20 g/ 4 fl. oz.) in combination with vitamin D and other key nutrients, on 400 meter walk time in mobility-limited older adults.

The decision to study older individuals with demonstrated functional limitations is multi-factorial. We have established that reduced muscle mass and loss of muscle strength and power are strong independent predictors of the ability to perform several mobility-related tasks, such as walking and stair climbing.[61-65] Targeting individuals prior to the onset of clinically evident disability may have important public health implications for designing function promoting therapies to prevent mobility disability with advancing age. In addition, these “at risk” individuals may represent a cohort of older individuals where functional limitations in walking and other tasks are closely related to impairments such as sarcopenia, reduced muscle strength and power, which may maximize the benefits from nutritional and/or exercise interventions.

The synergistic effect of protein supplementation on muscle function following both acute and chronic exercise in young and middle-aged adults has been demonstrated.[18, 66] In addition, vitamin D supplementation has been researched for a variety of outcomes in many populations, including falls, [67,

68] fracture, [69-73] and bone health.[74] No clinical trials have investigated the effects of protein supplementation combined with vitamin D supplementation on physical activity induced changes in older adults.

In a recent study conducted by Pahor et al.,[9] it was found that structured, moderate intensity physical activity can prevent mobility loss in mobility-limited older adults. This study adds further novelty by examining the synergy of a nutritional supplement with physical activity. If the results from this study are positive, and it is shown that the nutritional supplement provides added benefit to the physical activity intervention, more specific recommendations regarding protein intake combined with exercise may be warranted. Additionally, these results may rationalize investigating the feasibility of implementing this type of physical activity and nutritional supplementation program into a community setting.

The design of this study is subject to some limitations. First, as with any multi-center study, there are inherent difficulties in coordination across sites, particularly with sites located in different countries. The logistics of conducting a clinical trial may differ across study sites, which can take a considerable effort to address. To overcome this obstacle, monthly teleconference calls were held to discuss study progress, inclusion and exclusion criteria, and other procedural difficulties that may have occurred at the study sites. In addition, an on-site training was held at SWE, in order to ensure that procedures for all study outcomes, as well as the intervention, were conducted in a standardized way. Further, a face-to-face meeting with the study staff from both sites was held annually, which was used as a forum to discuss study logistics, timelines, and the analysis plan. Second, there is limited data being collected on nutritional status and dietary intake in this study.

In conclusion, the results of this study will further define our understanding of the role of a nutritional supplement on whole body physical functioning and accrual of fat free mass in older individuals with objectively measured functional limitations. Evidence from this trial will provide further

insight as to the impact of nutritional supplementation and lasting impact on physical function, body composition, and strength among older adults. If the nutritional supplement in this study is found to be effective, it will in combination with physical activity provide another method for mobility-limited older adults to improve their health and physical abilities.

Acknowledgements

This work was supported by Nestlé Health Science, Vevey, Switzerland.

Financial Disclosures

Dr. Roger Fielding has served as a consultant to Nestle Health Science, and has received honoraria for previous speaking engagements. Professor Tommy Cederholm has received speaking engagement honoraries from Nestle Health Science.

References:

1. Guralnik, J.M., et al., *A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission*. J Gerontol, 1994. **49**(2): p. M85-94.
2. Manton, K.G. and J.W. Vaupel, *Survival after the age of 80 in the United States, Sweden, France, England, and Japan*. N Engl J Med, 1995. **333**(18): p. 1232-5.
3. (WHO), W.H.O., *Good health adds life to years: global brief for World Health Day 2012*. 2012.
4. Campbell, W.W., et al., *Increased protein requirements in elderly people: new data and retrospective reassessments*. Am J Clin Nutr, 1994. **60**(4): p. 501-9.
5. Campbell, W.W., et al., *The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle*. J Gerontol A Biol Sci Med Sci, 2001. **56**(6): p. M373-80.
6. Hughes, V.A., et al., *Longitudinal changes in body composition in older men and women: role of body weight change and physical activity*. Am J Clin Nutr, 2002. **76**(2): p. 473-81.
7. Chapman, I.M., et al., *The anorexia of ageing*. Biogerontology, 2002. **3**(1-2): p. 67-71.
8. Volpi, E., et al., *Basal muscle amino acid kinetics and protein synthesis in healthy young and older men*. JAMA, 2001. **286**(10): p. 1206-12.
9. Pahor, M., Jack M. Guralnik, Walter T. Ambrosius, Steven Blair, Denise E. Bonds, Timothy S. Church, Mark A. Espeland et al, *Effect of Structured Physical Activity on Prevention of Major Mobility Disability in Older Adults: The LIFE Study Randomized Clinical Trial*. JAMA, 2014.
10. Kukuljan, S., et al., *Effects of resistance exercise and fortified milk on skeletal muscle mass, muscle size, and functional performance in middle-aged and older men: an 18-mo randomized controlled trial*. J Appl Physiol (1985), 2009. **107**(6): p. 1864-73.
11. Holm, L., et al., *Protein-containing nutrient supplementation following strength training enhances the effect on muscle mass, strength, and bone formation in postmenopausal women*. J Appl Physiol (1985), 2008. **105**(1): p. 274-81.
12. Iglay, H.B., et al., *Resistance training and dietary protein: effects on glucose tolerance and contents of skeletal muscle insulin signaling proteins in older persons*. Am J Clin Nutr, 2007. **85**(4): p. 1005-13.
13. Rosendahl, E., et al., *High-intensity functional exercise program and protein-enriched energy supplement for older persons dependent in activities of daily living: a randomised controlled trial*. Aust J Physiother, 2006. **52**(2): p. 105-13.
14. Bunout, B., et al., *Effects of nutritional supplementation and resistance training on muscle strength in free living elders. Results of one year follow*. J Nutr Health Aging, 2004. **8**(2): p. 68-75.
15. Haub, M.D., et al., *Effect of protein source on resistive-training-induced changes in body composition and muscle size in older men*. Am J Clin Nutr, 2002. **76**(3): p. 511-7.
16. Esmarck, B., et al., *Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans*. J Physiol, 2001. **535**(Pt 1): p. 301-11.
17. Fiatarone, M.A., et al., *Exercise training and nutritional supplementation for physical frailty in very elderly people*. N Engl J Med, 1994. **330**(25): p. 1769-75.
18. Tang, J.E., et al., *Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men*. J Appl Physiol (1985), 2009. **107**(3): p. 987-92.
19. Hartman, J.W., et al., *Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters*. Am J Clin Nutr, 2007. **86**(2): p. 373-81.
20. Garlick, P.J., *The role of leucine in the regulation of protein metabolism*. J Nutr, 2005. **135**(6 Suppl): p. 1553S-6S.

21. Katsanos, C.S., et al., *A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly*. Am J Physiol Endocrinol Metab, 2006. **291**(2): p. E381-7.
22. Visser, M., et al., *Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam*. J Clin Endocrinol Metab, 2003. **88**(12): p. 5766-72.
23. Bischoff-Ferrari, H.A., et al., *Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y*. Am J Clin Nutr, 2004. **80**(3): p. 752-8.
24. Mowe, M., E. Haug, and T. Bohmer, *Low serum calcidiol concentration in older adults with reduced muscular function*. J Am Geriatr Soc, 1999. **47**(2): p. 220-6.
25. Bischoff, H.A., et al., *Muscle strength in the elderly: its relation to vitamin D metabolites*. Arch Phys Med Rehabil, 1999. **80**(1): p. 54-8.
26. Dhesi, J.K., et al., *Neuromuscular and psychomotor function in elderly subjects who fall and the relationship with vitamin D status*. J Bone Miner Res, 2002. **17**(5): p. 891-7.
27. Pfeifer, M., et al., *Effects of a short-term vitamin D and calcium supplementation on body sway and secondary hyperparathyroidism in elderly women*. J Bone Miner Res, 2000. **15**(6): p. 1113-8.
28. Stein, M.S., et al., *Falls relate to vitamin D and parathyroid hormone in an Australian nursing home and hostel*. J Am Geriatr Soc, 1999. **47**(10): p. 1195-201.
29. Uusi-Rasi, K., et al., *Exercise and vitamin d in fall prevention among older women: a randomized clinical trial*. JAMA Intern Med, 2015. **175**(5): p. 703-11.
30. Kenny, A.M., et al., *Effects of vitamin D supplementation on strength, physical function, and health perception in older, community-dwelling men*. J Am Geriatr Soc, 2003. **51**(12): p. 1762-7.
31. Grady, D., et al., *1,25-Dihydroxyvitamin D3 and muscle strength in the elderly: a randomized controlled trial*. J Clin Endocrinol Metab, 1991. **73**(5): p. 1111-7.
32. Rolland, Y.M., et al., *Reliability of the 400-m usual-pace walk test as an assessment of mobility limitation in older adults*. J Am Geriatr Soc, 2004. **52**(6): p. 972-6.
33. Guralnik, J.M., et al., *Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery*. J Gerontol A Biol Sci Med Sci, 2000. **55**(4): p. M221-31.
34. Guralnik, J.M., et al., *Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability*. N Engl J Med, 1995. **332**(9): p. 556-61.
35. Folstein, M.F., S.E. Folstein, and P.R. McHugh, *"Mini-mental state". A practical method for grading the cognitive state of patients for the clinician*. J Psychiatr Res, 1975. **12**(3): p. 189-98.
36. Chale, A., et al., *Efficacy of whey protein supplementation on resistance exercise-induced changes in lean mass, muscle strength, and physical function in mobility-limited older adults*. J Gerontol A Biol Sci Med Sci, 2013. **68**(6): p. 682-90.
37. Bandura, A., *Social foundations of thought and action*.
38. Rejeski, W.J., et al., *Older adults with chronic disease: benefits of group-mediated counseling in the promotion of physically active lifestyles*. Health Psychology, 2003. **22**(4): p. 414.
39. Borg, G., *Perceived exertion as an indicator of somatic stress*. Scand J Rehabil Med, 1970. **2**(2): p. 92-8.
40. Nelson, M.E., et al., *Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association*. Med Sci Sports Exerc, 2007. **39**(8): p. 1435-45.
41. Dawson-Hughes, B., et al., *IOF position statement: vitamin D recommendations for older adults*. Osteoporos Int, 2010. **21**(7): p. 1151-4.
42. Newman, A.B., et al., *Association of long-distance corridor walk performance with mortality, cardiovascular disease, mobility limitation, and disability*. JAMA, 2006. **295**(17): p. 2018-26.

43. Newman, A.B., et al., *Walking performance and cardiovascular response: associations with age and morbidity--the Health, Aging and Body Composition Study*. J Gerontol A Biol Sci Med Sci, 2003. **58**(8): p. 715-20.
44. Bassey, E.J., et al., *Leg extensor power and functional performance in very old men and women*. Clin. Sci., 1992. **82**: p. 321-327.
45. Visser, M., et al., *Validity of fan-beam dual-energy X-ray absorptiometry for measuring fat-free mass and leg muscle mass. Health, Aging, and Body Composition Study--Dual-Energy X-ray Absorptiometry and Body Composition Working Group*. J Appl Physiol (1985), 1999. **87**(4): p. 1513-20.
46. Clark, D.J., et al., *Impaired voluntary neuromuscular activation limits muscle power in mobility-limited older adults*. J Gerontol A Biol Sci Med Sci, 2010. **65**(5): p. 495-502.
47. Reid, K.F., et al., *Muscle power failure in mobility-limited older adults: preserved single fiber function despite lower whole muscle size, quality and rate of neuromuscular activation*. Eur J Appl Physiol, 2012. **112**(6): p. 2289-301.
48. Frontera, W.R., et al., *Muscle fiber size and function in elderly humans: a longitudinal study*. J Appl Physiol (1985), 2008. **105**(2): p. 637-42.
49. Goodpaster, B.H., et al., *Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study*. J Appl Physiol (1985), 2001. **90**(6): p. 2157-65.
50. Kelley, D.E., B.S. Slasky, and J. Janosky, *Skeletal muscle density: effects of obesity and non-insulin-dependent diabetes mellitus*. Am J Clin Nutr, 1991. **54**(3): p. 509-15.
51. Frontera, W.R., V.A. Hughes, and W.J. Evans, *Reliability of isokinetic muscle strength testing in 45 to 75 yr old men and women*. Arch. Phys. Med. Rehabil., 1993. **74**: p. 1181-1185.
52. Hamilton, G.F., C. McDonald, and T.C. Chenier, *Measurement of grip strength: validity and reliability of the sphygmomanometer and jamar grip dynamometer*. J Orthop Sports Phys Ther, 1992. **16**(5): p. 215-9.
53. Kaiser, M.J., et al., *Validation of the Mini Nutritional Assessment short-form (MNA-SF): a practical tool for identification of nutritional status*. J Nutr Health Aging, 2009. **13**(9): p. 782-8.
54. Radloff, L., *The CES-D scale a self-report depression scale for research in the general population*. Applied Psychological Measurement, 1977. **1**(3): p. 385-401.
55. McHorney, C.A., J.E. Ware, Jr., and A.E. Raczek, *The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs*. Med Care, 1993. **31**(3): p. 247-63.
56. Pruitt, L.A., et al., *Use of accelerometry to measure physical activity in older adults at risk for mobility disability*. J Aging Phys Act, 2008. **16**(4): p. 416-34.
57. Tai, M.M., *A mathematical model for the determination of total area under glucose tolerance and other metabolic curves*. Diabetes Care, 1994. **17**(2): p. 152-4.
58. Candow, D.G., et al., *Protein supplementation before and after resistance training in older men*. Eur J Appl Physiol, 2006. **97**(5): p. 548-56.
59. Tieland, M., et al., *Protein supplementation increases muscle mass gain during prolonged resistance-type exercise training in frail elderly people: a randomized, double-blind, placebo-controlled trial*. J Am Med Dir Assoc, 2012. **13**(8): p. 713-9.
60. Tieland, M., et al., *Protein supplementation improves physical performance in frail elderly people: a randomized, double-blind, placebo-controlled trial*. J Am Med Dir Assoc, 2012. **13**(8): p. 720-6.
61. Baumgartner, R.N., et al., *Epidemiology of sarcopenia among the elderly in New Mexico*. Am J Epidemiol, 1998. **147**(8): p. 755-63.
62. Delmonico, M.J., et al., *Alternative definitions of sarcopenia, lower extremity performance, and functional impairment with aging in older men and women*. J Am Geriatr Soc, 2007. **55**(5): p. 769-74.

63. Janssen, I., S.B. Heymsfield, and R. Ross, *Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability*. J Am Geriatr Soc, 2002. **50**(5): p. 889-96.
64. Lauretani, F., et al., *Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia*. J Appl Physiol (1985), 2003. **95**(5): p. 1851-60.
65. Melton, L.J., 3rd, et al., *Epidemiology of sarcopenia*. J Am Geriatr Soc, 2000. **48**(6): p. 625-30.
66. Hartman, J.W., et al., *Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters*. Am J Clin Nutr, 2007. **86**(2): p. 373-81.
67. Bischoff, H.A., et al., *Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial*. J Bone Miner Res, 2003. **18**(2): p. 343-51.
68. Bischoff-Ferrari, H.A., et al., *Effect of Vitamin D on falls: a meta-analysis*. JAMA, 2004. **291**(16): p. 1999-2006.
69. Chapuy, M.C., et al., *Effect of calcium and cholecalciferol treatment for three years on hip fractures in elderly women*. BMJ, 1994. **308**(6936): p. 1081-2.
70. Chapuy, M.C., et al., *Vitamin D3 and calcium to prevent hip fractures in the elderly women*. N Engl J Med, 1992. **327**(23): p. 1637-42.
71. Chapuy, M.C., et al., *Combined calcium and vitamin D3 supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: the Decalyos II study*. Osteoporos Int, 2002. **13**(3): p. 257-64.
72. Dawson-Hughes, B., et al., *Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older*. N Engl J Med, 1997. **337**(10): p. 670-6.
73. Trivedi, D.P., R. Doll, and K.T. Khaw, *Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial*. BMJ, 2003. **326**(7387): p. 469.
74. Dawson-Hughes, B., et al., *Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older*. N Engl J Med, 1997. **337**(10): p. 670-6.