

Effect of Beetroot Juice on Body Composition, Physical Function, Muscle Strength, and Peak Oxygen Consumption in Resistance Trained Older Adults Consuming a Protein Supplement

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Abstract

Introduction: Body composition changes with aging, including reduction in lean body mass leads to impaired physical function, decreased muscular strength and reduced peak oxygen consumption (VO_{2peak}), and notably, increased mortality and loss of independence. The present study compared the effects of a nitrate containing beetroot juice supplement (BRJ+Nitrate) vs. a placebo (Control) on total and regional body fat free mass, quadriceps muscle strength, lower body mobility, and VO_{2peak} in healthy, older adults undergoing a 10-week long progressive resistance training program with protein supplementation.

Methods: In this double-blind, placebo-controlled trial healthy men and women (n=24) were randomized into BRJ+Nitrate or Control group. Measurements obtained at baseline and 10-week follow-up included whole and regional body composition, lower limb muscular strength, 400 meter walk time, stair climb time, and VO_{2peak} .

Results: No between group differences were seen for outcomes as analyzed using a 2-way analysis of variance. Improvements were apparent over time when both groups were combined in muscle strength, stair climb time, 400 meter walk time, and VO_{2peak} , but not in total or regional body composition measures.

Conclusions: The use of a nitrate containing beetroot juice does not enhance the improvement of muscle strength, physical function, and VO_{2peak} observed in healthy older adults undergoing a resistance training program coupled with protein supplementation.

Keywords: Nitrate; Insulin Resistance; Mobility Disability

Clinicaltrials.gov: NCT05460351

Introduction

Declines in lean body mass with aging leads to impaired physical function, decreased muscular strength and reduced peak oxygen consumption (VO_{2peak}), and notably, increased mortality and loss of independence [4, 32]. Thus, developing effective and safe interventions to prevent or delay the reduction in lean body mass in older adults is critical. In comparison to younger adults, older adults have lower rates of postprandial protein synthesis, and are less responsive to the effect of protein supplementation to acute protein synthesis [13]. This dampened response with protein supplementation is,

at least in part, due to an impaired insulin-stimulated endothelial-dependent vasodilation [34]. Insulin sensitive tissues, such as skeletal muscle, experience an increase in blood flow when nitric oxide is present, but no such response is observed when insulin resistant. This is present even in non-diabetic older adults with normal glucose tolerance and muscle glucose metabolism [11]. Thus, insulin resistance decreases the nitric oxide mediated vasodilator effect, which consequently reduces blood flow and the delivery of nutrients to the tissue [35]. Typically, the insulin response generated after the consumption of either carbohydrates or amino acids facilitates nitric oxide dependent vasodilation and subsequently, tissue perfusion. Consequently, the increase in the transport of amino acids into body tissues stimulates the mTORC1 pathway and protein synthesis in the tissue [18, 35].

One mechanism to increase nitric oxide in tissues is through the nitrate-nitrite-nitric oxide pathway [20]. Plasma nitrate is derived from endogenous mechanisms (including the oxidation of nitric oxide by nitric oxide synthase) and from dietary consumption (especially nitrate rich vegetables including beets and beet root juice) [12]. Plasma nitrate is concentrated in salivary glands and secreted back into the oral cavity where bacteria in the oral cavity partially reduce salivary nitrate to nitrite [9]. After swallowing, nitrate and nitrite in the gastrointestinal tract are absorbed into the plasma. Plasma nitrite is then reduced to nitric oxide, preferentially under hypoxic and acidic conditions such as in anaerobically active muscle tissue [9].

In a recent study insulin and sodium nitroprusside were infused in healthy older adults to investigate muscle perfusion and amino acid availability in non-diabetic older adults [34]. This treatment enhanced skeletal muscle protein synthesis through increased muscle perfusion and amino acid delivery as compared to insulin only treatment. As an alternative to using an organic nitrate such as sodium nitroprusside, consuming beetroot juice, a dietary source high in inorganic nitrate, increases nitric oxide bioavailability through the nitrate-nitrite-nitric oxide pathway. Previous research showed beetroot juice reduces resting blood pressure, lowers VO_2 levels during submaximal exercise, improves regional brain blood flow, and increases time to task failure during aerobic activities [17, 31].

Thus, the central supposition driving this work is that beetroot juice in combination with a protein supplement and resistance exercise training will improve muscle strength and physical function and increase skeletal muscle mass in older adults. The present study compared the effects of a nitrate containing beetroot juice supplement (BRJ+Nitrate) vs. a nitrate depleted beetroot juice placebo (Control) on fat free mass, quadriceps muscle strength, lower body mobility, and VO_{2peak} in healthy, older adults who consumed a protein supplement and participated in a 10-week long progressive resistance training program. This was of interest in that It was hypothesized that the BRJ+Nitrate intervention would result in a greater increase in lean body mass, quadriceps muscle strength, physical function, and VO_{2peak} following a 10-week

resistance training program supplemented by protein in comparison to the Control intervention. The rationale for the addition of the protein supplement was based on the premise that the mechanism for the improvement in body composition, physical function, and muscle strength from the nitrate containing beetroot juice would be due to improved blood flow to the working tissues, principally skeletal muscle. The protein supplement was provided to enhance substrate delivery, i.e. amino acids, to the muscles for anabolic and recovery of the tissue.

Materials & Methods

Subjects

In this double-blind, placebo-controlled trial, individuals were randomized into one of two groups: nitrate containing beetroot juice (BRJ+Nitrate) or nitrate depleted beetroot juice (Control). The study was approved by the Institutional Review Board and all participants provided written informed consent prior to their participation. Community-dwelling, non-diabetic, non-resistance training, older (≥ 60 years) men and women (n=24) were recruited to participate in this study. Interested individuals completed an initial telephone screen which included the collection of age, current medications, basic medical exclusion criteria such as medical diagnoses, confirmation of menopause in women, physical activity assessment and willingness to participate in the 10-week training program and consume the beetroot juice and whey protein supplements. If they passed the initial phone screen, they were scheduled to report to the laboratory to undergo further baseline testing. Prior to collection of measures at the baseline laboratory visit, participants completed an informed consent and a Health Insurance Portability and Accountability Act (HIPAA) authorization form. Full inclusion/exclusion criteria are shown in Table 1. Eligibility testing at the initial laboratory visit included measurement of height, weight, fasting glucose (to rule out diabetes), taste test of the beetroot juice and protein supplement, and graded exercise test.

Individuals interested in participating (n=145) were initially screened for eligibility by telephone. Thirty subjects were deemed eligible and interested in the study, and they all completed baseline testing visit 1. Twenty-seven subjects completed testing at baseline visit 2 with a total of 24 individuals randomized into the two intervention groups (see CONSORT flow diagram, Figure 1). Exclusions following baseline testing at visits 1 and 2 included: scheduling conflicts (n=2), inability to complete all testing and training (n=2), injury outside of the study (n=1), and abnormal response to graded exercise treadmill test (n=1). Two subjects withdrew from the Control group during the study unrelated to the intervention: one for knee surgery and one for heart surgery. One person withdrew from the BRJ+Nitrate group after randomization due to lack of time. One adverse event (severe dehydration) occurred during the intervention session. A total of 21 subjects completed the 10 week follow-up visit (n=12 for BRJ+Nitrate and n=9 for Control).

Table 1. Inclusion and exclusion criteria

Inclusion Criteria	
1.	≥60 years of age
2.	Able to provide own transportation to study testing visits and intervention
3.	Able to read and write in English
4.	Nondiabetic, i.e. fasting glucose < 126 mg/dl
5.	Sedentary (less than 60 minutes of moderate intensity structured physical activity each week and occurs in no more than 10 minute blocks and not participating in a resistance exercise training program)
6.	Willingness to provide informed consent and participate in intervention. Participants with diagnosed depression were permitted to participate provided that they have been medically stable without medication change for at least 3 months.
Exclusion Criteria	
1.	Smoking or use of chewing tobacco
2.	Involved in another intervention research study
3.	Diabetic (type 1 or 2)
4.	Atrophic gastritis
5.	Hypo- or hyperthyroidism
6.	Gout or history of kidney stones
7.	History of hypotension
8.	Premenopausal (women)
9.	Aversion to study-related testing procedures
10.	Allergy/sensitivity/aversion to beetroot beverages or protein supplement
11.	Medical conditions with contraindications for engaging in resistance training program or taking the protein supplement
12.	Systemic, uncontrolled diseases (diabetes, recent (last 6 months) or current treatment of cancer, thyroid disorders, cardiovascular disease, COPD, or inflammatory bowel diseases)
13.	Use of any of the following medications: phosphodiesterase type 5 inhibitors, nitroglycerin or nitrate preparations, proton pump inhibitors, or medications for hypothyroidism
14.	Potential inability to complete the tasks required for the protocol as well as conditions that may interfere with interpretation of results including: inability to ambulate, severe congestive heart failure or severe cardiovascular disease, neoplasm for practical and neurophysiologic reasons
15.	Individuals with contraindications for performing a graded exercise test or have medical contraindications for participating in a scheduled exercise training program
16.	Individuals more than 300 pounds or less than 125 pounds due to DEXA weight limits and possibility of exceeding recommended daily nitrate dose of 7 mg/kg body weight, respectively.

Table 2. Baseline characteristics. Values for age, body mass, and body mass index are presented as means ± standard deviation, Values for race and gender are shown as frequency and percent of sample.

Variable	BRJ+Nitrate(n=13)	Control (n=11)	p value
Age, yrs	67.7 ± 6.5	66.9± 6.2	p=0.803
Gender- female, n (%)	10 (76.9%)	7 (70%)	p=0.476
Race – white, n (%)	11 (84.6%)	10 (90.9%)	P=0.642
Body Mass, kg	72.9± 13.9	81.8± 18.7	p=0.530
Body Mass Index, kg/m ²	28.1 ± 5.6	28.9 ± 5.4	p=0.704

Testing and Measurements

Testing of the primary and secondary aims occurred prior to randomization at baseline and following the 10-week exercise and nutrition intervention. Testing took place on 2 separate days at each time point with the graded exercise test occurring on the first day and body composition and physical function tests measured on the second testing day.

Body weight and height: Using standard techniques, body weight and height were assessed to calculate body mass index.

Whole body and regional body composition: Whole body composition (fat mass and fat free mass) was acquired using total body dual energy x-ray absorptiometry (DXA) (GE Healthcare iDXA, General Electric Medical Systems Lunar, Madison, WI, USA) with the participant supine and aligned with the scanner table as prescribed by the manufacturer. Regional body composition (volumes of thigh muscle, thigh intermuscular fat, subcutaneous fat, and visceral fat) was assessed using computed tomography (CT) (LightSpeed Plus™, General Electric Medical Systems, Milwaukee, WI, USA). For the abdominal region, the inner and outer aspects of the abdominal wall were manually traced. Visceral fat was defined as fat enclosed by the inner aspect of the abdominal wall and subcutaneous fat defined as the fat outside the outer aspects of the abdominal wall. For the thigh, intermuscular fat and visible intermuscular adipose tissue was separated from subcutaneous adipose by drawing a line along the deep fascial plane surrounding the mid-thigh muscles. The total volume of fat within the measured scans was used as a measure of fat volume (cm³) and the average value across both legs was used in data analyses. Total mid-thigh muscle volume was also assessed by CT (cm³).

Lower extremity muscle strength: Quadriceps muscle peak torque was measured using a Biodex System 4 Pro isokinetic dynamometer (Biodex Medical Systems, Shirley, NY, USA) at a speed of 60° per sec. Hips and knees were positioned at 90° of flexion and the input axis of the dynamometer was aligned with each the lateral epicondyle of the involved leg. Dynamometer position was recorded for each subject to match baseline and follow-up positions. Subjects were asked to extend the knee as hard as possible against the resistance pad, with a stop angle of 30° of flexion. The best performance of 3 trials was used for analyses.

400m walk: In this test, participants walked 10 laps in a corridor marked by cones on a 20 meters long indoor course. Participants were instructed to do their best and to walk at a

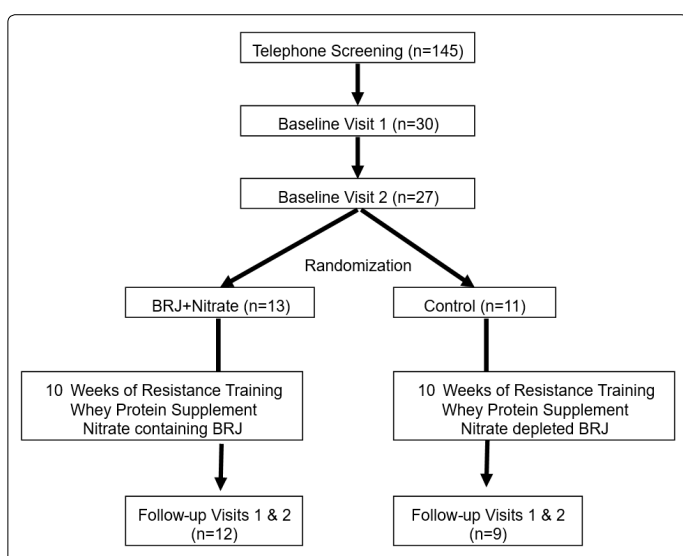


Figure 1. CONSORT Flow Diagram

No significant differences were found among groups for baseline characteristics (Table 2). Across the cohort, mean age was about 67 years with 70% women and 90% white. Initial body mass index was approximately 28 kg/m².

fast pace; they were also given standardized encouragement during the testing. Performance was measured as time in minutes and seconds to the nearest 0.1 seconds.

Stair climb: The 12 Stair Climb was conducted on an indoor set of 12 stairs. Subjects were instructed to move as fast as they safely could and to give their best effort. The time to ascend and descend the 12 stairs was recorded to the nearest 0.01 seconds.

Graded exercise testing: A physician-supervised graded exercise test was performed using an individualized treadmill ramp protocol until volitional exhaustion. Subjects were then asked to select a brisk walking pace based on their own fitness and comfort level. Speed remained the same throughout the test. Grade was set at 0% for the first 2 min and was increased 1% every 30 seconds thereafter. Expired gas analysis was performed on a breath-by-breath basis during exercise using a Medical Graphics Ultima™ Cardio₂® gas exchange analysis system (Medical Graphics Diagnostics Corporation, Saint Paul, MN, USA). All subjects received verbal encouragement throughout the test. This test was completed at baseline and 10-weeks follow-up to obtain peak oxygen uptake (VO_{2peak}).

Dietary intake: Daily food logs were used to assess dietary protein intake throughout the 10-week study. Protein content of foods was based on the American Diabetes Association food exchanges. Additionally, at each exercise visit, participants received a list of high nitrate containing foods to avoid and they designated on the form if they consumed any of these foods, and the amount and frequency consumed if they did.

Intervention Components

Resistance exercise training and supplementation: The resistance exercise training program was identical across both treatments and consisted of a center-based individualized, progressive resistance program. It was 3 days/week, 60 minutes/session with a goal of 3 sets of 8-12 repetitions on the main muscle groups for upper and lower body movements at 80% of predetermined maximal strength. Exercises performed on Nautilus® (Vancouver, Washington, USA) weight machines included: leg press, hip abduction, knee extension, knee flexion, seated row, vertical chest press, and incline chest press. Starting resistance for each session was determined from the one repetition maximum (RM) testing at the initial training visit. The intensity of the training was increased gradually over 2 weeks to reach the study goal. At week 1, intensity was 1 set at 10-12 repetitions of 50% of 1 RM. By the end of week 2, intensity was progressed to 2 sets at 8-12 repetitions of 70% of 1 RM. When participants were able to complete 3 sets of 12 repetitions at two consecutive training sessions, the resistance was increased to maintain progressive overload on the muscles. Study staff instructed participants on performing the exercises through the full range of motion in ~0.5 seconds for concentric contractions and 1-2 seconds for eccentric contractions. Records were kept for session attendance. Training volume (resistance, repetitions, and sets for each station) was documented.

Following each 60-minute resistance training session, participants were given one, 70 ml bottle of BEET IT Sport

Shot (James White Drinks Ltd; Suffolk, United Kingdom) (with or without nitrate depending upon randomization) in addition to a 15 g serving of the whey protein supplement (GNC® Pro Performance 100% Whey Protein) with instructions to consume both within a 15-minute period at the training facility. The whey protein contained 15 g of leucine. The beetroot juice for both BRJ+Nitrate and Control was supplied in a 70 ml serving (Beet It Shot®, James White Drinks, Suffolk, United Kingdom). The placebo (Control) was indistinguishable in appearance and taste from the BRJ+Nitrate. As measured in our lab, the BRJ+Nitrate contained 380 mg (6.1 mmol) of nitrate per dose of supplement; the Control group consumed the same volume of juice supplement with the nitrate removed from the beverage by the manufacturer. Each shot contained 72 kcals with 2.5 g protein and 16 g of carbohydrates in a serving of the supplement. The BRJ+Nitrate and Control and protein supplements were provided and consumed at the exercise facility to facilitate compliance to the intervention. Furthermore, participants were instructed to avoid high nitrate sources in their diet (see section below).

Diet: Participants were given a list of high nitrates containing foods and told to limit/avoid these during the days when the supplement is consumed. All participants were requested to maintain a protein intake of 1.0 g/kg of body weight. This is a level that at a minimum provides the Dietary Reference Intakes of 0.8 g/kg body weight; research has also shown that this intake results in a net balance of zero in fat free mass in older adults undergoing a resistance training program [7]. All participants were counseled on their habitual diet consumption to meet the study goals. Each participant had an individualized daily protein intake goal and were provided with instructions and sample diets using food group exchange lists on how to meet this goal. Participants kept a food record and used food group exchanges to and calculate daily protein intake. These were reviewed weekly by the interventionists and participants were provided with suggestions for dietary manipulations on how to achieve their protein goal. The protein content of the whey and beetroot juice supplements was included in the daily protein goal.

Data analysis: Differences in baseline characteristics and compliance data between groups were analyzed using independent t-tests and data are reported as means ± standard deviation; race and gender were analyzed using chi-square tests. A 2-way analysis of variance (ANOVA) was used - with group as the between factor and time as the within factor - to test for differences in primary and secondary outcomes, as well as in weekly workloads. Results from this analysis are reported as estimated marginal means ± standard error of the mean. Statistical significance was accepted at p < 0.05. All analyses were performed using SPSS® version 28.0 (Chicago, IL).

Results

Adherence to exercise intervention and supplement use: When examining compliance to the intervention, no significant difference was found between BRJ+Nitrate and Control in the number of exercise sessions attended (~95% for both groups;

28.3 ± 0.7 vs 28.7 ± 0.5 sessions, respectively out of 30 possible sessions). The range for attendance was 21 to 30 sessions for completers, with 17 individuals attending at least 29 of the 30 sessions. Additionally, all participants consumed both the protein supplement and their respective beetroot juice at every exercise session attended.

Knee extensor strength and physical function: Results for knee extensor strength and physical function assessments are shown in Table 3. No group*time interactions and no group differences over the intervention period were observed in any of these measures. However, when groups were combined to study the effect of the strength training intervention in conjunction with the protein supplement over time, knee extensor strength increased from baseline to follow-up (Time means: 107.0 ± 9.0 (Baseline) vs 115.0 ± 10.5 (10-Week) Nm; p=0.022). Similarly, when groups were combined time to climb the stairs was statistically significantly faster at follow-up compared to baseline (Time means: 4.7 ± 0.3 (Baseline) vs. 4.4 ± 0.3 (10-Week) secs; p=0.050). There was a trend (p=0.083) for the time to complete the 400-meter walk to be lower (faster walking pace) at follow-up (Time means: 4.4 ± 0.2 minutes) than baseline (Time means: 4.6 ± 0.2 minutes).

Table 3. Physical function, maximal oxygen consumption, and body composition (total and regional) at baseline and 10-week follow-up for completers only. Values are LSmeans ± standard error of the mean for baseline and 10-Week values. The Time Means and Treatment Means are LSmeans± standard error of the mean.

Variable	BRJ+Nitrate	Control	Time Means
Knee Extensor Strength, Nm			
Baseline	100.9 ± 11.7	113.2 ± 13.7	107.0 ± 9.0
10-Week	107.7 ± 13.6	122.2 ± 15.9	115.0 ± 10.5*
Treatment Means	104.3 ± 12.5	117.7 ± 14.7	
Stair Climb Time, secs			
Baseline	5.0 ± 0.4	4.4 ± 0.5	4.7 ± 0.3
10-Week	4.7 ± 0.4	4.2 ± 0.4	4.4 ± 0.3*
Treatment Means	4.8 ± 0.4	4.3 ± 0.4	
400 Meter Walk Time, min			
Baseline	4.9 ± 0.3	4.3 ± 0.3	4.6 ± 0.2
10-Week	4.7 ± 0.3	4.1 ± 0.3	4.4 ± 0.2#
Treatment Means	4.8 ± 0.3	4.2 ± 0.3	
Peak Oxygen Consumption, ml O ₂ /kg/min			
Baseline	20.7 ± 1.5	22.5 ± 1.7	21.6 ± 1.1
10-Week	22.1 ± 1.6	24.1 ± 1.8	23.1 ± 1.2*
Treatment Means	21.4 ± 1.5	23.3 ± 1.7	
Peak Oxygen Consumption, ml O ₂ /min			
Baseline	1497 ± 123	1687 ± 143	1592 ± 94
10-Week	1614 ± 140	1816 ± 161	1715 ± 107*
Treatment Means	1556 ± 130	1752 ± 150	
Total Lean Body Mass, kg			
Baseline	43.1 ± 2.7	46.1 ± 3.4	44.6 ± 2.2
10-Week	43.6 ± 3.0	46.8 ± 3.6	45.2 ± 2.3
Treatment Means	43.4 ± 2.8	46.4 ± 3.5	
Total Fat Mass, kg			
Baseline	29.0 ± 2.4	27.8 ± 3.0	28.4 ± 1.9
10-Week	28.9 ± 2.3	27.6 ± 2.8	28.2 ± 1.8
Treatment Means	29.0 ± 2.4	27.7 ± 2.9	
Thigh Muscle Volume, cm ³			
Baseline	1082 ± 80	1203 ± 93	1142 ± 61
10-Week	1095 ± 90	1167 ± 104	1130 ± 68
Treatment Means	1089 ± 82	1184 ± 94	

Thigh Fat Volume, cm ³			
Baseline	1642 ± 128	1425 ± 148	1534 ± 98
10-Week	1654 ± 122	1425 ± 141	1540 ± 93
Treatment Means	1648 ± 124	1425 ± 144	
Thigh Subcutaneous Fat Volume, cm ³			
Baseline	1535 ± 128	1329 ± 148	1432 ± 98
10-Week	1559 ± 117	1326 ± 135	1443 ± 89
Treatment Means	1547 ± 122	1327 ± 141	
Abdominal Fat Volume, cm ³			
Baseline	2636 ± 276	2289 ± 319	2463 ± 211
10-Week	2616 ± 260	2298 ± 300	2457 ± 199
Treatment Means	2626 ± 268	2294 ± 309	
Abdominal Visceral Fat Volume, cm ³			
Baseline	687 ± 96	545 ± 111	616 ± 73
10-Week	675 ± 93	537 ± 107	606 ± 71
Treatment Means	681 ± 94	541 ± 108	
Abdominal Subcutaneous Fat Volume, cm ³			
Baseline	1827 ± 201	1621 ± 232	1724 ± 154
10-Week	1816 ± 193	1634 ± 223	1725 ± 147
Treatment Means	1821 ± 197	1628 ± 227	

VO₂peak: There were no group*time interactions and no group differences over the intervention period for VO₂peak expressed in relative or absolute terms (Table 3). However, when collapsed across groups VO₂peak (expressed both as ml O₂/kg body weight/min and ml O₂/min) showed significant increases of 7-8%, indicating improvement in cardiovascular fitness for the cohort.

Total and regional body composition: There was no statistically significant group* time interactions and no group or time main effects for any body composition assessments (Table 3). This includes total body fat free mass and fat mass (as measured by DXA) and regional fat free mass and fat mass (as measured by CT images).

Discussion

This study tested the hypothesis the BRJ+Nitrate intervention would result in a greater increase in fat free mass, quadriceps muscle strength, physical function, and VO₂ peak following a 10-week resistance training program supplemented by protein in comparison to the Control intervention. The underlying mechanism for this hypothesis was that the insulin-resistance that occurs with aging, even in the presence of normal glucose homeostasis, dampens post-prandial protein synthesis, and that dietary nitrate would enhance nitric oxide dependent vasodilation and improve nutrient delivery to the muscles to increase resistance training benefits on physical function, body composition, and cardio-respiratory fitness in older adults. The results from the study do not support our hypothesis as there were no group differences in these measures. However, when groups were combined there was a main effect of time on several of the outcomes, including knee extensor muscle strength, stair climb time, 400-meter walk time and VO₂peak.

Improvements in measures of skeletal muscle strength, physical function, and cardiovascular fitness after 10-weeks of resistance training plus protein supplementation were apparent across both intervention groups. We observed a

7-8% significant increase in knee extensor strength following the intervention. The increase in muscle strength is consistent with previous research, although others showed up to 20% changes in strength [1, 2, 25]. It is speculated that the lower anabolic response observed is, at least in part, due to insulin resistance apparent with aging, even in the presence of normoglycemia. Similarly, the improvement in physical function assessments with resistance training is consistent with earlier research. [2]

In contrast, to improvements in strength and physical function, there were no changes over time for body composition assessments, either total body or regional measurements. The effect of resistance training on body composition in older adults, particularly accumulation of lean body mass, is equivocal. Consistent with our findings, others found no significant effects on lean body mass after resistance training in older adults [1, 33]. One contributing factor to the lack of significant lean body mass gain is likely the duration of the resistance training intervention. Studies demonstrating favorable body composition adaptation often have durations of ≥ 12 weeks, with some interventions lasting ≥ 1 year [22, 25]. Two meta-analyses examining resistance training adaptations in older adults reported a mean training period of at least 4-5 months to achieve increases in lean body mass. This is nearly twice the length of our current study. Another potential contributing factor to the lack of gain in lean mass is the inadequate total energy intake during the resistance training intervention. Nicklas et al [28] and a recent meta-analysis [27] found impaired lean body mass gains in resistance trained subjects who were also being energy restricted. An energy deficit of ~ 500 kcal/day prevented significant gain to lean mass in older individuals [27]. Our study did not assess daily energy intake, and this was not emphasized to participants as the main focus was on protein intake and the beetroot juice supplement.

The addition of a protein supplementation to a resistance training program is a strategy employed to enhance the adaptations from resistance training, particularly on strength, body composition, and physical function [8, 22]. A meta-analysis aimed at assessing changes in strength and body composition variables in response to ≥ 6 weeks of resistance training with or without protein supplementation found that when performing resistance training, conjunctive protein supplementation outperformed resistance training alone in lean body mass gain and fat mass loss [26]. However, In a sub-analysis controlling for age, they report non-significant gain in lean body mass for those over 45 years.

Similar to the percent change of knee extensor muscle strength, VO_2 peak increased 7-8 percent from baseline to follow-up. There are mixed results from previous research with some showing significant increases [19] and little to no changes demonstrated by others [16]. The magnitude of change in VO_2 peak we observed is consistent with the 5-8% increase previously reported [19]. In contrast, Karavirta et al. [16] found smaller nonstatistical increases of 3-5% in VO_2 peak

after 12-26 weeks of resistance training. While resistance training effects on VO_2 peak are more modest compared to aerobic training, [21] they potentially are important considering the 5-15% decline in VO_2 max that occurs each decade after age 25 years. It is estimated one-third to one-half of the decline in VO_2 max from 30 to 70 years old is attributed to loss of muscle mass [10, 30]. By increasing the skeletal muscle mass, improvement in VO_2 max and VO_2 peak may be apparent. Thus, one method for eliciting improvements in VO_2 max may be through strength training.

The reason for the lack of differences between group responses is unknown. However, potential considerations include length of the training sessions, dosage of the supplements, and efficacy of converting dietary nitrate into plasma nitrite. Session attendance to the exercise training was high with $>90\%$ attendance for both groups; furthermore, only 3 participants attended fewer than 29 of the 30 scheduled sessions. Consumption of the protein and beetroot juice supplements was 100% at each attended exercise session. Thus, poor adherence to the interventions is not likely an explanation for the lack of intervention effect.

Importantly, the dosage of nitrate from the beetroot juice source used was 380 mg (6.1 mmol) per 70 ml bottle. This dose of nitrate, although on the lower end of therapeutic effectiveness, has demonstrated physiological actions on blood flow, blood pressure, exercise capacity, and oxygen consumption [3, 5, 14, 29]. This dose was chosen as it provides a total daily intake of nitrate below the United States Environmental Protection Agency daily intake level of 7.0 mg (0.11 mmol) nitrate /kg body weight. Thus, a higher dose of nitrate may be needed to elicit a response in this cohort as a dose-dependent response has been observed with blood pressure [15]. Consideration must also be given to the resistance-training program, specifically its length and progression. In a meta-analysis examining dose-response of resistance training in older adults, a longer training period may be needed [6]. Older adults may require a higher protein intake than generally recommended for younger adults. The current dietary recommendation for all adults is 0.8 g of protein/kg of body weight/day [21]. The current investigation prescribed a protein intake of 1.0 g/kg of body weight/day. However, Volpi et al. found older adults have a reduced anabolic response to a given amount of protein [36]. This is consistent with the finding showing older adults require 1.0 to 1.5 g/kg of body weight of protein per day [37]. Therefore, a higher daily protein intake may be needed for older adults, particularly when undergoing a resistance training exercise program. Due to laboratory technical issues, plasma nitrate and nitrite were not assessed in this study. This is a limitation as the mechanism for the proposed improvement in the outcomes is based on an increase in nitric oxide metabolism through the nitrate-nitrite-nitric oxide pathway. Previous research in our laboratory using Beet It Shot products has shown increases in plasma nitrate and nitrite levels [23, 24].

Conclusion

In conclusion, these results suggest that a 10-week resistance training program coupled with protein supplementation increased muscle strength, physical function, VO_2 peak in healthy older adults. The addition of a nitrate containing beetroot juice does not add to the effect. This finding may have practical utility for exercise prescription amongst the healthy older adult population. Endurance training is the most common training method used to improve VO_2 peak. However, resistance training should be considered a viable option for improving strength, physical function, and VO_2 peak in a population known to have declines in these measures.

Conflicts of Interest

The authors declare no conflict of interest.

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Author Contributions

Conceptualization: GDM and MJB; Formal analysis: GDM and MJB; Funding Acquisition: GDM; Investigation: GDM and JTB; Methodology: GDM; Project Administration: GDM, JTB, and MJB; Writing Original Draft: GDM; Writing Review and Editing: GDM, JMM and MJB

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