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Daily Consumption of Polyphenol-rich Grape Powder Improves Muscle Strength Markers in Postmenopausal Women

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Abstract

Sarcopenia is a debilitating age-associated condition with no effective treatment options. Therefore, there is high interest in evaluating whether food components, such as polyphenols found in grapes, could mitigate sarcopenia. However, the exact influence of grapes on age-related muscle loss in humans remains unknown. Herein, we conducted a pilot double-blind parallel clinical dietary intervention trial with 15 healthy postmenopausal women who consumed, either freeze-dried grape powder or a control powder once daily for 6 weeks. We observed that women consuming grape powder had significantly increased hand grip scores, a measurement of muscle strength, from baseline to week six compared to the control group. Additionally, women consuming grape powder significantly improved their performance on the gait speed test over the six-week period. Furthermore, the age-adjusted model revealed that the grape group completed the gait speed test significantly faster, compared to the control group. Mechanistically, we assessed plasma irisin concentrations. Although, they did not reach statistically significance, when comparing the change from baseline to week six, the grape group presented a 14.4% ($p=0.07$) increase in plasma irisin levels, while the control group had a 7.8% decrease ($p=0.08$). Moreover, we observed a positive significant association between changes in grip strength and changes in irisin (from baseline to Week 6), when adjusting for group and age, suggesting that the increase in grip strength in the grape group may be due to increases in irisin levels. In summary, daily consumption of grape powder favorably modulated grip strength and gait speed, two parameters to assess sarcopenia, warranting further evaluation.

Keywords: Sarcopenia, muscle strength, grapes, polyphenols, irisin

Introduction

Sarcopenia is a debilitating age-related condition that can lead to severe health issues. It is estimated that in 2017, over 50 million people were suffering from sarcopenia worldwide, and in the next 40 years, this condition is expected to affect over 200 million people [1]. Sarcopenia is linked with fragility, lack of endurance, physical inactivity, slow gait speed, and increased morbidity and mortality. Several studies have shown higher prevalence of sarcopenia in women then in men [2]. In a recent study, it was shown that sarcopenia was associated with higher risk of death from all causes compared to non-sarcopenic subjects in ages >60 years old and more prevalent in ages >80 years old [3]. Clearly, sarcopenia is an emerging public health issue with a huge socioeconomic burden, with limited treatment options.

Multiple mechanisms have been shown to contribute to the development of sarcopenia [4]. The major ones include: i) chronic low-grade inflammation, which can accelerate muscle degradation, ii) oxidative stress, which can damage muscle cells and impairs their ability to regenerate protein metabolism; iii) hormonal changes, in which the decline of anabolic hormones, such as testosterone, growth hormone, and insulin-like growth factor-1 (IGF-1) contribute to reduced muscle protein synthesis; and iv) neuropathic factors, where loss of motor neurons can lead to muscle atrophy and weakness [4].

Another proposed mechanism includes the regulation of irisin, a myokine that is reduced in sarcopenic subjects [5]. For instance, sarcopenic patients had significantly lower circulating irisin levels. Additionally, they found that a serum irisin concentration below 1 ng/mL is associated with a 95% risk of developing sarcopenia. Moreover, Huh et al. have shown a positive correlation between circulating insulin-like growth factor 1 and irisin, which suggests the possibility that irisin could be involved in exercise-induced muscle hypertrophy [6]. Furthermore, some recent studies have indicated that circulating irisin can signal skeletal muscle growth [7-9]. The above evidence suggests that irisin could be an important modulator of sarcopenia.

Given the clinical significance of sarcopenia, ongoing research is exploring dietary interventions to mitigate muscle loss in the elderly. Phenolic compounds present in grapes include flavanols (catechin and epicatechin), anthocyanins (peonidin, delphinidin, cyanidin, malvidin), flavonols (kaempferol, isorhamnetin, quercetin), and resveratrol. These phenolic compounds, known for their anti-inflammatory, anti-carcinogenic, and antioxidative properties, have been linked to muscle health [10, 11]. For example, quercetin reduces inflammation and fibrosis, epicatechin can increase markers of muscle growth, and resveratrol prevents muscle atrophy by maintaining mitochondrial function [12-16]. Moreover, in a recent preclinical study, a four-week dietary supplementation with freeze-dried grape powder modulated the expression of genes related to muscle health in the skeletal muscle of aged mice compared to a control diet [17], suggesting improved muscle function. In addition, preclinical evidence indicates that polyphenols can modulate irisin levels. For instance, phenolic compounds present in a grape pomace extract (GPE), prevent irisin downregulation in rats fed a high-fat diet [18]. However, whether the daily consumption of grapes and their polyphenolic compounds can mitigate sarcopenia in humans remains unknown.

The goal of this study was to evaluate the role of daily grape consumption in mitigating sarcopenia parameters and key metabolic regulators, with a possible implication of irisin. For this purpose, 15 postmenopausal women were randomized into a double-blind, two-arm, parallel dietary intervention trial and asked to consume 46 g of freeze-dried grape powder or a control for six weeks. Hand grip strength was measured before and after the dietary intervention as a measure of sarcopenia risk. Circulating irisin concentrations were also measured from blood samples collected from participants enrolled in the dietary intervention trial.

Materials and Methods

Participants

Healthy postmenopausal women aged 60 years or older, with a hand grip strength value of 21 kg or lower, body mass index (BMI) values between 18.4 and 24.9 kg/m2, blood pressure below 130/80 mmHg, and fasting plasma glucose concentration below 100 mg/dl were recruited from the greater Sacramento, California area. Exclusion criteria included specific muscle diseases, peripheral vascular disease, intermittent claudication, central and peripheral nervous system disorders, cachexia, active diagnosis of diabetes mellitus, myocardial infarction, stroke, liver disease, severe infection, anemia, undergoing dialysis or long-term steroid therapy, active cancer treatment, use of blood pressure, blood glucose, or blood cholesterol medications, and blood donations in the past 30 days. Additionally, individuals were excluded if there were smokers, followed a vegan or vegetarian diet and were unwilling to limit consumption of certain dietary supplements and foods high in polyphenols.

Study Design

The pilot study was a randomized, double-blind parallel two arm study that evaluated the effects of consuming freeze-dried grape powder or low polyphenolic control powder daily, for six weeks, on parameters of sarcopenia in post-menopausal women. This study was conducted between March 2023 and March 2024 at the University of California, Davis (UCD) Ragle Human Nutrition Center. It was registered at ClinicalTrials.gov (NCT05863507), with the protocols approved by the UCD Institutional Review Board (IRB #1867416) Social & Behavioral Committee, ensuring ethical compliance. All participants provided their written informed consent for inclusion before participating in the study.

Participants were recruited through Davis Enterprise Newspaper advertisements, UC Davis email lists, posting flyers on social media (Nextdoor, Instagram, and Facebook), and at community centers, grocery stores, local senior centers, and retirement communities. Recruitment and enrollment followed the Consolidated Standards of Reporting Trials (CONSORT) strategy (**Figure 1**). Interested individuals underwent a telephone interview to assess their potential eligibility for

the study and to receive information about its design and procedures Individuals who met the basic study inclusion and exclusion criteria were asked to attend an in-person screening visit (Visit 0) following a 12-hour fast. After informed consent was obtained, their grip strength, anthropometrics, and blood pressure were recorded. Health status, dietary habits and physical activity levels were assessed with questionnaires, and fasting glucose levels were checked using a finger-prick blood sample (True Metrix, Trividia health, FL, USA).

A total of 15 eligible participants were enrolled and randomized to consume either grape feezed-dried powder or control powder daily for 6 weeks. One participant from the control group withdrew from the study due to cramping joints. Outcome measures were collected after an overnight fast at baseline visit (Visit 1), after 3 weeks (Visit 2) and 6 weeks (Visit 3) of consuming study products.

Participants were asked to maintain their regular diets and physical activity levels throughout the study but limit the consumption of polyphenol-rich food to no more than ½ cup a day of blueberries, strawberries, raspberries, blackberries, bilberries, huckleberries, cherries, grapes, and pomegranate, no more than 3 cups a day of green and black tea and coffee, no more than 1 glass per day of red wine, and no more than 10 g per day of dark chocolate. Adherence was monitored by 3-day food records (two work days and one weekend day) and 7-day physical activity questionnaires.

Freeze-dried grape powder and control

The freeze-dried grape powder and the low polyphenolic control powder were provided by the California Table Grape Commission. The grape powder was a composite of fresh red, green and black California grapes (seeded and seedless varieties), that was frozen, ground with food-quality dry ice, freeze-dried, and re-ground using Good Manufacturing Practices for food products throughout. The nutrient content is provided in **Supplemental Table 1**. The powder was

processed and stored in the -80C freezer to preserve the integrity of the biologically active compounds found in fresh grapes. The total polyphenol content in grape powder was 163 mg of gallic acid equivalent (GAE) per 46g of powder. It contained resveratrol, flavanols (including catechin), flavonols (including quercetin), anthocyanins and simple phenolics (**Supplemental Table 2**).

The low polyphenolic control powder was formulated to closely match the freeze-dried grape powder in terms of calories, sugar profile, organic acid profile, as well as for sensory characteristics of sweetness, tartness, mouthfeel, and viscosity. The nutrient content is provided in **Supplemental Table 1**. It contained fructose, glucose (as dextrose), the two main sugars in the grape powder, organic acids including tartaric, malic and citric acids. Artificial colors (FD&C dyes) were used to replace natural color components and avoid any addition of polyphenolic compounds from natural colorants. All flavorings were free of polyphenolic compounds and antioxidants. The total polyphenol content in control powder was less than 46 mg of GAE per 46g of powder. The control also contained modified food starch and tapioca maltodextrin, two potassium salts, and silicon dioxide at the same level as used in the grape powder.

Participants consumed either 46g of freeze-dried grape powder or control powder daily for 6 weeks. The powders were stored in the freezer until consumed to preserve the integrity of the biologically active compounds. Each powder was resuspended in around 160 ml (6oz) of water and consumed all at once as a drink. Compliance was tracked using calendar logs filled out by participants, which recorded marked dates of consumption, as well as through the return of used powder packets.

The amount of grape powder consumed is equivalent to 1½ cups of fresh grapes a day and falls within the recommended intake range (46-69 grams per day) for human studies according to the California Table Grape Commission.

Outcome Measures

Hand Grip strength

The hand grip test was conducted using the Jamar Plus Digital Hand Dynamometer (Performance Health Supply, WI, USA). The participants were instructed to squeeze the dynamometer as firmly as possible three times per hand, alternating hands after each measurement. The test was performed while seated, with both feet flat on the ground, and the arm positioned at a 90-degree angle without support from armrests, tabletops, or any other forms of support. The average value of recorded grip strength measurements (kg) was used for the analyses.

Short Physical Performance Battery

Short physical performance battery (SPPB), comprised of balance, gait speed and repeated chair stand tests, was used to evaluate lower extremity function including balance, mobility, and strength.

For the balance test, participants were asked to perform three unassisted stances for 10 seconds each: a feet-together stand (feet placed side by side), a semi-tandem stand (one foot slightly in front of the other while touching), and a tandem stand (one foot placed directly in front of the other). If the participants stepped out of the position, their time under 10 seconds was recorded.

For the gait speed test, participants were asked to walk a 4-meter distance at their normal walking speed, from behind the start marker to the end marker, indicated by tape on the ground. They were also advised not to slow down before passing the end mark. Time was recorded when their first foot crossed the end marker, and the fastest time from two trials was used for analysis.

The chair stand test was performed with participants standing up from a seated position while keeping their arms crossed against the chest. After confirming their ability to perform one

full sit-to-stand action, participants were asked to repeat this movement 5 consecutive times, as quickly and safely as possible, and the total time was recorded in seconds.

For all tests, lab personnel were always standing near the participants to ensure their safety. Points for each test were awarded appropriately based on the standard SPPB protocol.

Biochemical and hematological parameter analyses

For plasma isolation, whole blood was collected into PST lithium heparin or EDTA tubes and centrifuged for 15 min at 1600×g at 4°C immediately after collection. Lithium heparin plasma was analyzed at the UC Davis Medical Center to assess: fasting glucose, lipid panel [triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL)], kidney function (blood urea nitrogen (BUN), creatinine, sodium, potassium, chloride, and calcium), and liver function parameters (alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin and albumin).

EDTA plasma samples were used to measure irisin (Cat#: DY9420-05), insulin (Cat#: DINS00), and total adiponectin (Cat#: DHWAD0) levels, by ELISA (following the manufacturer's instructions (R&D Systems, MN, USA).

Fresh blood collected into EDTA tubes was used to assess the complete blood count by the UC Davis Medical Center.

Statistical Analysis

Repeated measure mix model ANOVA analysis was used to assess all variables over time. Violation of the assumption of sphericity was assessed using Mauchly's test, and the Greenhouse-Geisser statistic was used to determine the p-value. Sidak's multiple comparison test was used to identify significant pairwise comparisons between groups/timepoints.

For the main longitudinal outcomes (grip strength value, gait speed test, chair stand test, and irisin level) measured repeatedly at 3 and 6 weeks within the same participant, gamma mixed-

effects models with log links were fitted. The models included fixed effects of age (centered at 70 years), group (grape; control), time at testing (3, 6 weeks), the interaction between group and time, an offset term of log baseline outcome at Week 0 (before intervention start), and a random intercept to account for within-subject correlation. For the average grip strength value and irisin, the interactions were removed in the final models due to non-significance. For the gait speed test and chair stand test, the interactions between group and time were significant and remained in the models. We then estimated the percentage changes in the outcomes from baseline to Week 3 (or 6) for a 70-year-old participant in each group, by calculating the ratios of Week 3 (or 6) to Baseline outcome means minus 1, where the ratios were estimated by exponentiating the linear predictors (excluding the offset) in the fitted models. Based on the fitted models, we also reported the estimated ratios of grape to control in Week 6 vs baseline ratios (i.e., ratio of $grape_{week\ 6}/grape_{baseline}$ to $control_{week\ 6}/control_{baseline}$), where ratio>1 means that the grape group has higher percentage increase in the outcome compared to the control group, while ratio<1 means that the grape group has more percentage decrease in the outcome compared to the control group. In addition, we evaluated the association between change in average grip strength and change in irisin using linear regression, where the outcome was the percentage change of average grip strength value from baseline to Week 6, and covariates included age, group, percentage change of Irisin levels from baseline to Week 6, and interaction between group and percentage change of irisin. The interaction was removed in the final model due to non-significance. These models were fitted in SAS version 9.4 (SAS Institute, Cary, NC) and R version 4.4.3 (R Foundation for Statistical Computing, Vienna, Austria), and a two-sided alpha of 0.05 was used to determine statistical significance.

To assess the between-group differences at the end of the study (Week 6) relative to the baseline (Week 0), selected variables were analyzed by Welch's unpaired t-test or Mann-Whitney test. Data were visualized using GraphPad Prism version 10.3 (San Diego, CA, USA).

Results

Baseline characteristics

Fourteen women (11 Caucasian, 1 African American, 1 American Indian, and 1 South Asian) completed the study. Analysis of metabolic and lipid parameters, blood pressure, height, weight, and physical performance tests included all participants except one due to data that was out of range for multiple parameters. This resulted in the inclusion of seven individuals in the control group and six in the grape group. Baseline characteristics of study participants are provided in **Table 1**.

Effect of daily grape consumption on hand grip strength

Hand grip strength was measured to evaluate the effects of grape powder on muscle strength. Only in women consuming grape powder, there was a significant increase in hand grip strength, at both Week 3 (16.0±2.7 vs 18.3±2.9, p=0.011) and Week 6 (16.0±2.7 vs 18.5±2.3, p= 0.006) of consumption (**Figure 2A**). Moreover, by the end of the study, unlike in the control group, grip strength improved in all subjects consuming grape powder. However, due to the observed interindividual variability in response (ranging from 0.3 to 40.3% change from baseline), this effect was not statistically significant compared to the control group (p=0.11) (**Figure 2B**).

The observed significant effect of grape consumption on grip strength remained after adjusting for age, using a gamma mixed-effects model with a log link that included fixed effects of age (centered at 70 years). In the grape group, there was a significant increase in grip strength values from baseline to Week 3 (estimated: 14.63%, p=0.004) and Week 6 (estimated: 15.79%, p=0.003) (**Supplemental Table 3** and **Table 2**). In contrast, there were no significant changes in grip strength values observed in the control group from baseline to mid-point or endpoint of the study (**Table 2, Supplemental Table 3**).

Consistently, the Week 6 to baseline grip strength ratio comparing grape vs. control was significant (**Table 3**), suggesting grape group has higher percentage increase compared to the control.

Effect of daily grape consumption on physical performance

In order to evaluate physical performance over the course of the study, a set of SPPB tests was performed including balance, chair stands, and gait speed. When comparing combined final scores of these SPPB tests all subjects throughout the study scored in high range (9-12). Therefore, we focused our analyses on the individual tests.

Throughout the study, all participants were able to perform a feet-together stand and a semi-tandem stand for 10 seconds. Similarly, subjects were mainly successful in completing the tandem stand test. At Week 6 two subjects in both grape and control groups failed to maintain tandem stance for 10. Still, no differences were observed between groups.

In the grape group, the time required to complete the chair stand test tended to increase at the 3-week time point ($p=0.078$); however, this increase was significantly reduced by the end of the study ($p <0.0001$) (**Figure 3A**). Similar changes over time were also observed in an age-adjusted model, with a significant increase in time needed to complete the chair stand test at Week 3 but not at week 6 (**Table 2 and Supplemental Table 3**). Nevertheless, there was no significant difference between the grape and control groups in the time needed to complete the chair stand test relative to baseline by the end of the study (**Figure 3B, Table 3**).

When analyzing gait speed, we did not observe significant changes in the time required for subjects to complete the four-meter walk throughout the study within or between groups (**Figure 3C, D**). However, in a model adjusted by age, the estimated percent change over six weeks showed a significantly faster completion time for the four-meter walk (estimate: -14.7%, $p=0.021$) only in the grape group (**Table 2**). Moreover, the estimated grape to control ratio for gait

speed test at Week 6 tended to be significant (p=0.06) (**Table 3**), suggesting an improvement in muscle health following grape consumption.

Effect of daily grape consumption on irisin levels

To evaluate potential mediators of the effects of grape powder on sarcopenia, we assessed plasma irisin concentration. Although we observed changes in the irisin levels over the study duration, the effect did not reach statistical significance (p=0.076) (**Figure 4A**). At six weeks, the average irisin levels change from baseline between the grape and control group tended to be different, with the observed 14.4±24.0% increase in the grape group and 7.8±14.6% decrease in the control group (p=0.08) (**Figure 4B**). In the age-adjusted model there was a significant decrease in irisin levels only in control group at Week 3 (estimate: -21.2%, p=0.013), however this decreasing effect did not remain significant by Week 6 (**Supplemental Table 3** and **Table 2**). Similarly, to non-adjusted model, the differences between groups were not significant.

Moreover, we observed a positive significant association between change in grip strength and change in irisin (from baseline to Week 6), when adjusting for group and age (**Table 4**), showing a trend that the intervention "pushed" the correlation values toward top right direction (**Figure 4C**), suggesting that the increase in hand grip strength in the grape group may be due to increases in irisin levels.

Effect of daily grape consumption on adiponectin, insulin, and glucose levels

Circulating peptide hormones, adiponectin, and insulin are linked with glucose uptake into the muscle, affecting its function. Since their levels can be dysregulated in sarcopenia, we evaluated the effects of grape power on these parameters. There were no significant effects on plasma levels of these parameters over the six-week intervention period (**Figure 5 A, C, E**). Additionally, we observed no significant differences in percentage changes in these parameters between the grape and control groups from baseline to the end of the study (**Figure 5 B, D, F**).

Effect of daily grape consumption on lipid parameters

To examine the effects of grape consumption on lipid parameters, we assessed plasma levels of fasting total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides. Triglyceride plasma levels significantly changed over time (main effect p=0.006), with a trend toward an increase by Week 6, observed in a Control group (p=0.05). Over the duration of the study, there were no significant differences in lipid parameters, both within and between the grape and control groups (**Table 5**).

Effect of daily grape consumption on parameters of kidney and liver function

We assessed several plasma parameters to evaluate kidney function, including waste clearance (blood urea nitrogen and creatinine) and electrolyte balance (serum sodium, potassium, chloride, and calcium). Additionally, liver function was assessed by measuring albumin and bilirubin levels, as well as liver enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). All individuals in both the control and grape groups remained within the healthy range for these biomarkers at all time points (**Supplemental Table 4**). Throughout the six-week period, no significant differences were observed in the assessed parameters, either within or between the grape and control groups (**Supplemental Table 4**).

Effect of daily grape consumption on hematological parameters

Finally, we also assessed the effects of dietary intervention with grape powder or control on hematological parameters during six weeks. All individuals in both the control and grape groups remained within the optimal range for these hematological parameters at all time points (**Supplemental Table 5**), with no significant effects of grape consumption observed.

Discussion

Muscle mass loss is common in women due to the natural aging process and is associated with a lack of endurance, physical inactivity, slow gait speed, and decreased mobility. A potential nutritional strategy could be to consume foods high in polyphenols, like grapes, berries, and cherries, which may help. The findings of this pilot trial suggest that the daily intake of high-polyphenolic grape powder mitigates some sarcopenia parameters.

The European Working Group on Sarcopenia in Older People (EWGSOP) recommends handgrip strength and the chair stand test to assess muscle strength. Regarding hand grip strength, the EWGSOP proposed using low muscle strength, with a cut-off of <16 kg, measured by hand grip strength, as the primary criterion for diagnosing sarcopenia. However, many studies use a pre-sarcopenia range of 16-21 kg for women. Park H.S. et al. suggested alternative cut-offs for hand grip strength in older women: 14.77 kg for sarcopenia and 19.22 kg for pre-sarcopenia [5]. A 2022 meta-analysis classified sarcopenia with cut-offs of <20 kg (EWGSOP) and <18 kg (AWGS). Based on this, we chose a hand grip strength of 21 kg for inclusion criteria in this study, covering both sarcopenic and pre-sarcopenic individuals. A major finding of our study was that a daily consumption of grape powder, but not control powder, significantly improved grip strength over six weeks.

As a locomotor capacity, gait speed is representative of neuromuscular quality and a critical determining factor for healthy aging [19]. Indeed, the EWGSOP has developed an algorithm including gait speed measurement as the easiest and most reliable way to determine sarcopenia in clinical practice [20]. Similarly, the loss of muscle mass and the subsequent decline in gait speed are associated with aging. For instance, in a 4-year follow-up study of older Chinese adults, the percentage decline in gait speed was 8.2% for men and 9.0% for women [21]. Our findings suggest that the daily consumption of grape powder significantly improved gait speed by -14.7%, which may indicate enhanced muscle health and potential sarcopenia mitigation [22].

Previous animal studies suggest polyphenols have a positive effect on muscle health [13, 18]. For example, polyphenols have been shown to have anti-inflammatory properties and assist in repairing and regenerating skeletal muscle [10, 14, 23]. Moreover, dietary supplementation with freeze-dried grape powder modulated the expression of genes in the skeletal muscle of aged mouse [17], suggesting improved muscle function. We observed that compared to a low polyphenolic control powder, the daily consumption of grape powder improved two parameters used to assess muscle health. We observed a 15.79% change (from baseline) in hand grip strength in the grape group after six weeks. Additionally, in an age-adjusted model, gait speed significantly improved, with a 14.7% faster completion time for the four-meter walk. This suggests grape powder can improve muscle strength and potentially alleviate sarcopenia. Consistent with our results, (-)-epicatechin, found in grape powder, positively impacts muscles by increasing capillaries and muscle fiber mitochondria [15].

Irisin can be secreted into the circulation from exercising skeletal muscle, subcutaneous and visceral adipose tissue and then can travel to the brain, adipose tissue, bones, and pancreas. There is limited research on the physiology of irisin, but it has been suggested that PCC-1α is an upstream regulator of irisin and induces the browning of subcutaneous adipose tissue through uncoupling protein 1 (UCP-1) [24]. The regulation of circulating active irisin, a myokine known to regulate skeletal muscle growth [7-9], could represent a mechanism by which grapes may mediate sarcopenia prevention. Indeed, a preclinical study in rats has shown that polyphenols have the ability to modulate irisin levels [18]. In our pilot study, although the irisin changes were not statistically significant, the grape group showed a 14.4% increase in plasma irisin levels from baseline to week six, while the control group experienced a 7.8% decrease. The lack of significance could potentially be due to a lack of statistical power, the wide variation among individual participants' irisin levels, or differences in the thyrometabolic status of the participants, since irisin levels have been shown to be influenced by thyroid hormones [25]. Of note, changes in

irisin (from baseline to Week 6) positively correlated with the changes in hand grip strength. This aligns with previous findings by Park et al showing that circulating irisin was positively correlated with muscle mass as measured by quadricep cross-sectional area per body weight [5]. Our correlation findings suggest that the increase in grip strength in the grape group may be due to increases in irisin levels. However, the assotiation between irisin and grip strength was not strong, which could potentially be due to the high variability in irisin levels.

While this double-blind study provides novel insights in the effects of grape consumption on muscle function in humans, a main limitation of the study was the small number of participants in each group. Furthermore, an important consideration in the analysis of the obtained results is the amount of grape powder used. Participants consumed 46 grams of grape powder per day, equivalent to 1.5 cups of whole grapes, to assess its effect on grip strength and irisin levels. However, it is unknown whether this amount is enough to see an effect on irisin levels or grip strength since this research field is very limited. Another limitation was the lack of a detailed dietary assessment, as some of the sarcopenia parameters could be influenced by other nutrients. Although participants were instructed to maintain their habitual diet and limit the consumption of certain polyphenol-rich foods, we can not rule out the potential impact of other nutrients on the aforementioned sarcopenia parameters. Additionally, rather short duration of the study (six weeks) may not have been enough to fully capture the magnitude of the effect of grape powder on assessed parameters. Longer-term study with larger sample size would be necessary to validate findings from our study and allow better understanding of the effects of grape powder on muscle function. In a longer-term study, it would also be beneficial to assess these parameters with participants consuming the grape powder beverage during each visit and taking a baseline blood sample before consumption and a second sample after consuming the drink. This would allow for comparison of the acute effect, long-lasting effect as well as combined effect of both. Finally, this pilot study focused on postmenopausal women. However, including both sexes in

future research would help determine whether sex-specific responses exist in relation to grape powder supplementation.

In summary, results from this double-blind pilot study support the concept that daily intake of modest amounts of grape powder improves muscle strength maintenance in postmenopausal women. Future studies with larger sample sizes, longer duration and potentially a higher amount of grape powder (containing a higher dose of polyphenols) are needed to validate these results.

CRediT Author Contributions:

JLM: Methodology, Visualization, Investigation, Writing- Original draft preparation, Reviewing and editing. **CRL:** Conceptualization, Methodology, Investigation, Reviewing, and editing. **ZQ** and **SC**: Statistical analysis. Reviewing and editing of manuscript. **IK:** Methodology, Investigation, Statistical analysis, Visualization, Reviewing and editing. **GGM:** Conceptualization, Methodology and Investigation, Writing- Original draft preparation, Reviewing and editing of manuscript; Funding acquisition.

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Data availability: The data supporting this article have been included as part of the Supplementary Information.

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Table 1. Baseline characteristics of study participants

	Grape (n=6)	Control (n=7)
Baseline Characteristics		
Age (years)	70.5 ± 8.5	71.0 ± 5.9
Height (cm)	162.4 ± 4.4	166.4 ± 6.4
Weight (kg)	54.5 ± 3.1	60.3 ± 3.9
BMI (kg/m2)	20.7 ± 1.8	21.8 ± 1.3
Fasting glucose (mg/dl)	91.0 ± 6.1	90.9 ± 2.9
SBP (mmHg)	103.2 ± 9.8	118.2 ± 5.1
DBP (mmHg)	66.5 ± 6.1	72.9 ± 6.7

SBP, Systolic blood pressure, DBP, Diastolic blood pressure.
Data presented as Mean ± Standard deviation

Table 2. Estimated percentage changes of the primary interested outcomes from baseline for each group, based on random intercept gamma models.

Outcome	Grape		Control	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Estimated percentage change from Baseline to Week 6 for a 70-year old participant (%)				
Average Grip Strength Value	**15.79 (7.69, 24.51)**	**0.003**	3.56 (-3.23, 10.84)	0.336
Gait Speed Test	**-14.70 (-23.91, -4.41)**	**0.021**	0.68 (-9.45, 11.96)	0.902
Chair Stand Test	5.73 (-5.04, 17.73)	0.333	-6.67 (-15.55, 3.14)	0.206
Irisin Level	7.26 (-8.14, 25.25)	0.396	-5.11 (-18.17, 10.04)	0.504

Abbreviations: CI = Confidence Interval
The results were based on gamma mixed-effects models with log link that included fixed effects of age (centered at 70 years), group (Grape; Control), time at testing (3, 6 weeks), the interaction between group and time, an offset term of log baseline outcome at Week 0, and a random intercept to account for within-subject correlation. For Average Grip Strength Value and Irisin Levels, the interaction was removed due to non-significance. The estimated percentage changes in the outcomes were calculated by (ratios of Week 3 (or 6) to Baseline means) - 1, where the ratios were estimated by exponentiating the linear predictors (excluding the offset) in the fitted models for a 70-year-old participant.

Table 3. Estimated ratios of Grape to Control in Week 6 vs Baseline ratios for primary interested outcomes, based on random intercept gamma models adjusted for age.

Estimated ratios of Grape to Control[1]	Average Grip Strength Value (kg)		Gait Speed Test (sec)		Chair Stand Test (sec)		Irisin Level (pg/ml)	
	Ratio (95% CI)	***P***	**Ratio (95% CI)**	***P***	**Ratio (95% CI)**	***P***	**Ratio (95% CI)**	***P***
Week 3	**1.12 (1.01, 1.23)**	**0.041**	0.97 (0.83, 1.14)	0.683	**1.20 (1.04, 1.37)**	**0.038**	1.13 (0.93, 1.37)	0.228
Week 6	**1.12 (1.01, 1.23)**	**0.041**	0.84 (0.72, 0.99)	0.060	1.13 (0.98, 1.29)	0.123	1.13 (0.93, 1.37)	0.228

[1] Estimated ratios of Grape to Control in Week 6 vs Baseline ratios (i.e., ratio of $Grape_{week\ 6}/Grape_{baseline}$ to $Control_{week\ 6}/Control_{baseline}$) for primary interested outcomes, based on fitted gamma mixed-effects models with log link that included fixed effects of age (in years, centered at 70 years), group (Grape; Control), time at testing (3, 6 weeks), the interaction between group and time, an offset term of log baseline outcome at Week 0, and a random intercept to account for within-subject correlation. For Average Grip Strength Value and Irisin, the interaction between group and time were removed in the final model due to non-significance, and thus the reported group effects are the same across time. Ratio>1 means that the Grape group has higher percentage increase in the outcome compared to the Control group, while ratio<1 means that the Grape group has more percentage decrease in the outcome compared to the Control group.

Table 4. Parameter estimates from the liner regression model for percentage change of average grip strength value from baseline to Week 6 (%) to evaluate the association between change in average grip strength and change in irisin, adjusted by group and age.

Model term[1]	Estimate (*SE*)	*P*
Intercept	7.00 (4.49)	0.154
Percentage change of irisin from baseline to Week 6 (%)	**0.48 (0.21)**	**0.047**
Grape vs Control	4.28 (7.23)	0.569
Age (in years)	0.61 (0.51)	0.266

Abbreviations: SE = standard error.

[1] Estimated coefficients and *P*-values for model terms in linear regression models for percentage change of average grip strength value from baseline to Week 6 (%), including covariates of age (in years, centered at 70 years), group (Grape; Control), percentage change of Irisin levels from baseline to Week 6 (%), and interaction between group and percentage change of irisin. The interaction was removed in the final model due to non-significance. The coefficient of group can be interpreted as the direct effect of grape on the change of grip strength (i.e., excluding the indirect effect through the mediator irisin change).

Table 5. Plasma levels of lipid parameters over six weeks of dietary supplementation with grape powder or control

Variables	Grape			Control			p-value		
	Week 0	Week 3	Week 6	Week 0	Week 3	Week 6	t	I	t x I
CHO (mg/dl)	190.7 ± 16.3	199.5 ± 12.8	193.0 ± 19.3	212.9 ± 38.4	206.1 ± 41.5	218.4 ± 46.1	0.71	0.32	0.15
HDL-c (mg/dl)	78.7 ± 18.9	84.8 ± 17.7	77.0 ± 18.8	78.3 ± 17.1	76.7 ± 15.6	76.6 ± 16.6	0.12	0.76	0.07
LDL-c (mg/dl)	99.3 ± 14.0	103.6 ± 13.7	100.9 ± 11.8	119.5 ± 32.6	115.3 ± 36.3	126.3 ± 37.5	0.55	0.22	0.31
TG (mg/dl)	63.7 ± 7.5	55.2 ± 10.0	75.3 ± 10.9	55.5 ± 17.3	60.3 ± 17.3	70.3 ± 27.4#	**0.01**	0.75	0.25

TG, triglyceride; CHO, Total cholesterol, HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; p-values for the effect of time (t); effect of dietary intervention (I), and their interaction (txI), from repeated measure mixed analysis ANOVA. Data presented as Mean ± Standard deviation, # p=*0.052 at week 0 vs 6.*

FIGURE LEGENDS

Figure 1. CONSORT flow diagram of the study

Figure 2: Changes in hand grip strength. **A.** Grip strength over the duration of the study in subjects consuming control (blue) or grape powder (green), **B.** Percent change in grip strength at week six relative to baseline. Data are presented as mean ± SD. *, $p < 0.05$, **, $p < 0.01$.

Figure 3. Changes in time needed to complete physical performance tests. A. Chair stand time over six weeks for the control (blue) and grape (green) group. **B.** Percentage change in chair stand time at week six relative to baseline. **C.** Gait speed time over six weeks. **D.** Percent change in gait speed time at week six relative to baseline for both groups. Data are presented as mean ± SD. ****, $p<0.0001$.

Figure 4. Changes in irisin levels and their link with grip strength. A. Plasma irisin levels over the duration of the study in the control (blue) and grape (green) group. **B.** Percentage change in irisin levels at week six relative to baseline for both study groups. C. The significant positive relationship between changes in irisin levels and grip strength at week six, both expressed as a percentage change from baseline. Data are presented as mean ± SD.

Figure 5. Changes in plasma adiponectin, insulin, and glucose levels following six weeks of grape powder or control consumption. A. Plasma levels of adiponectin at baseline and week six. **B.** Percentage change in adiponectin in the grape and control groups. **C.** Plasma levels of

insulin at baseline and week six. **D.** Percentage change in insulin in the grape and control groups. **E.** Plasma levels of glucose at baseline and week six. **F.** Percentage change in glucose in grape and control groups. Data are presented as mean ± SD.

Data availability:

The data supporting this article have been included as part of the Supplementary Information

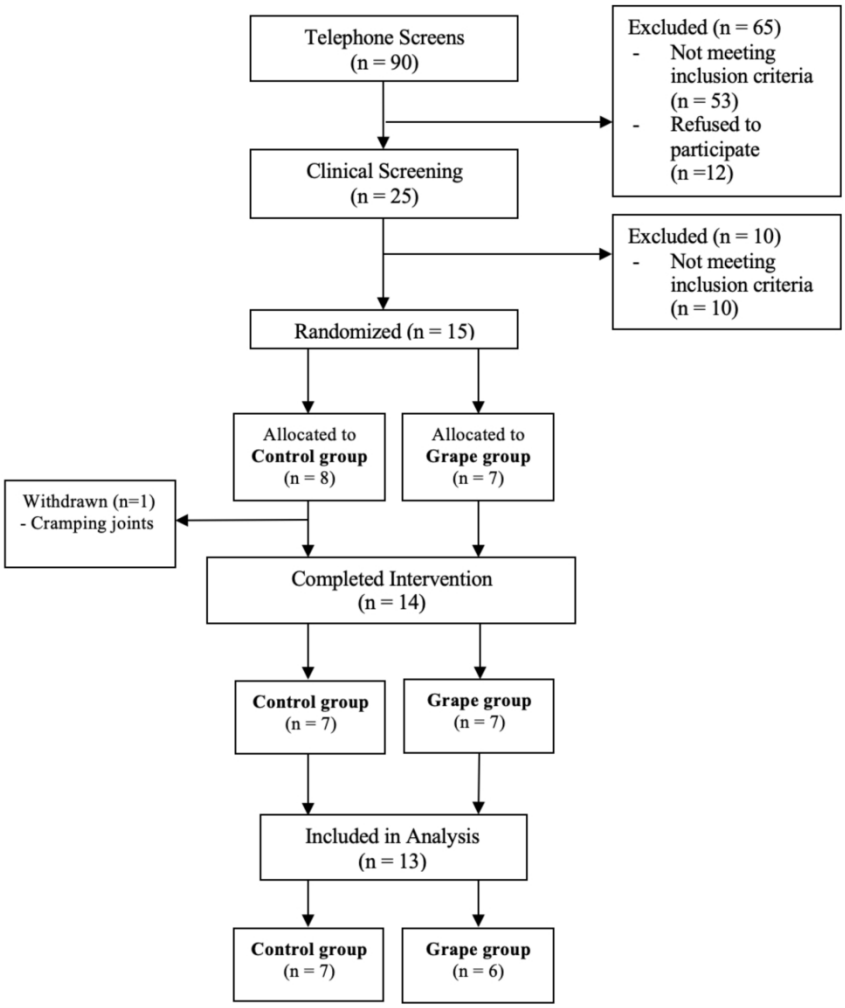

Figure 1

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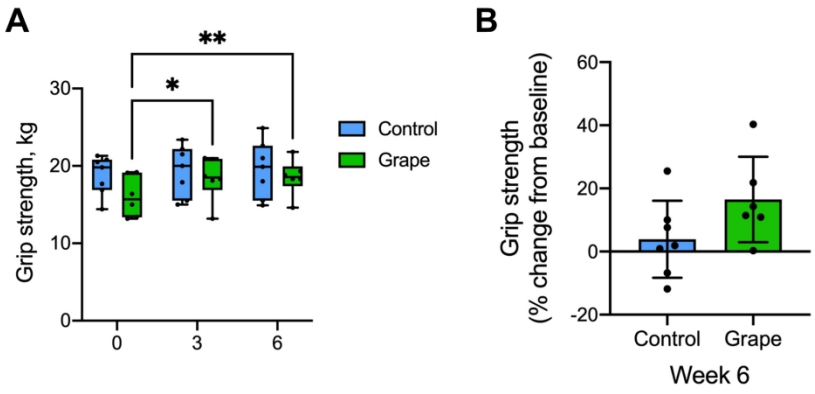

Figure 2

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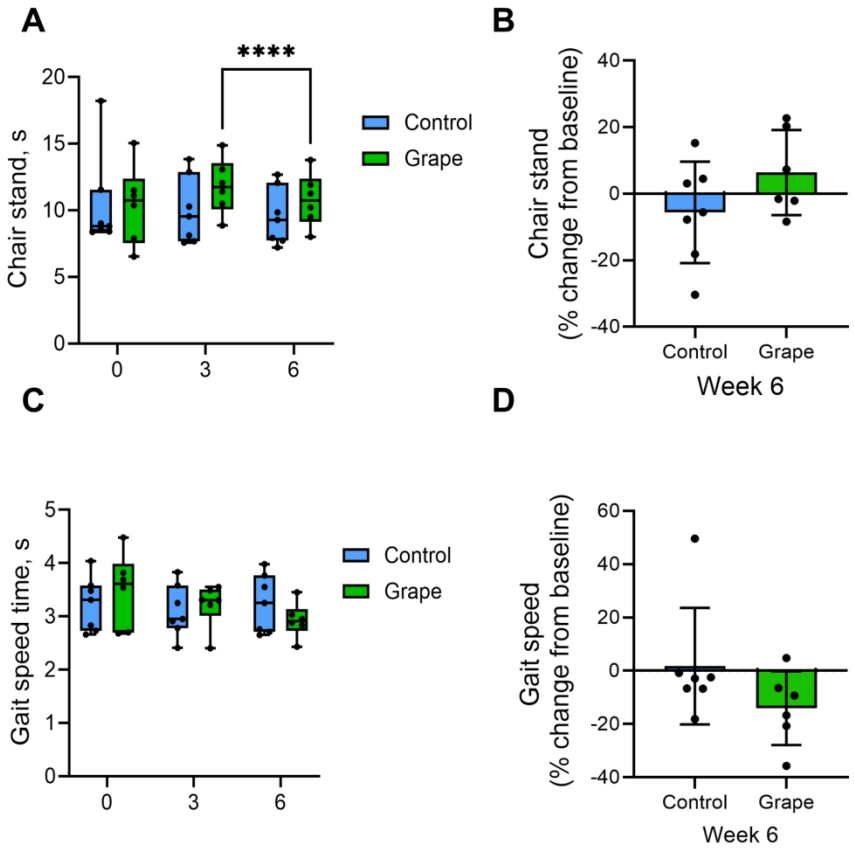

Figure 3

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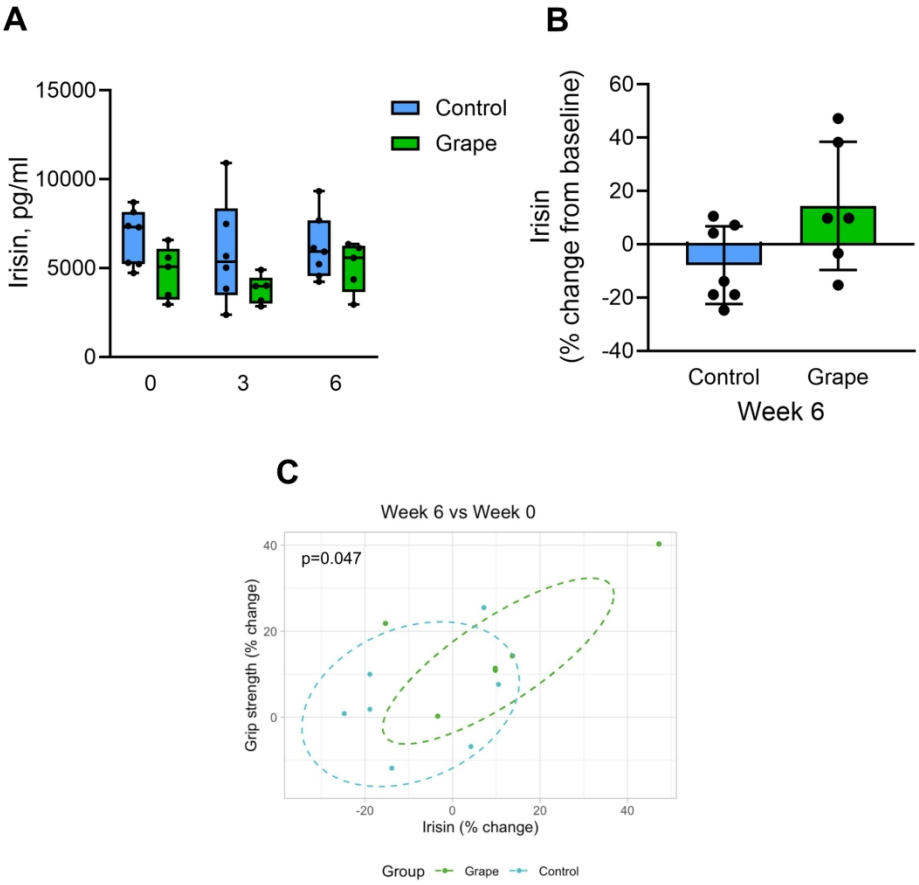

Figure 4

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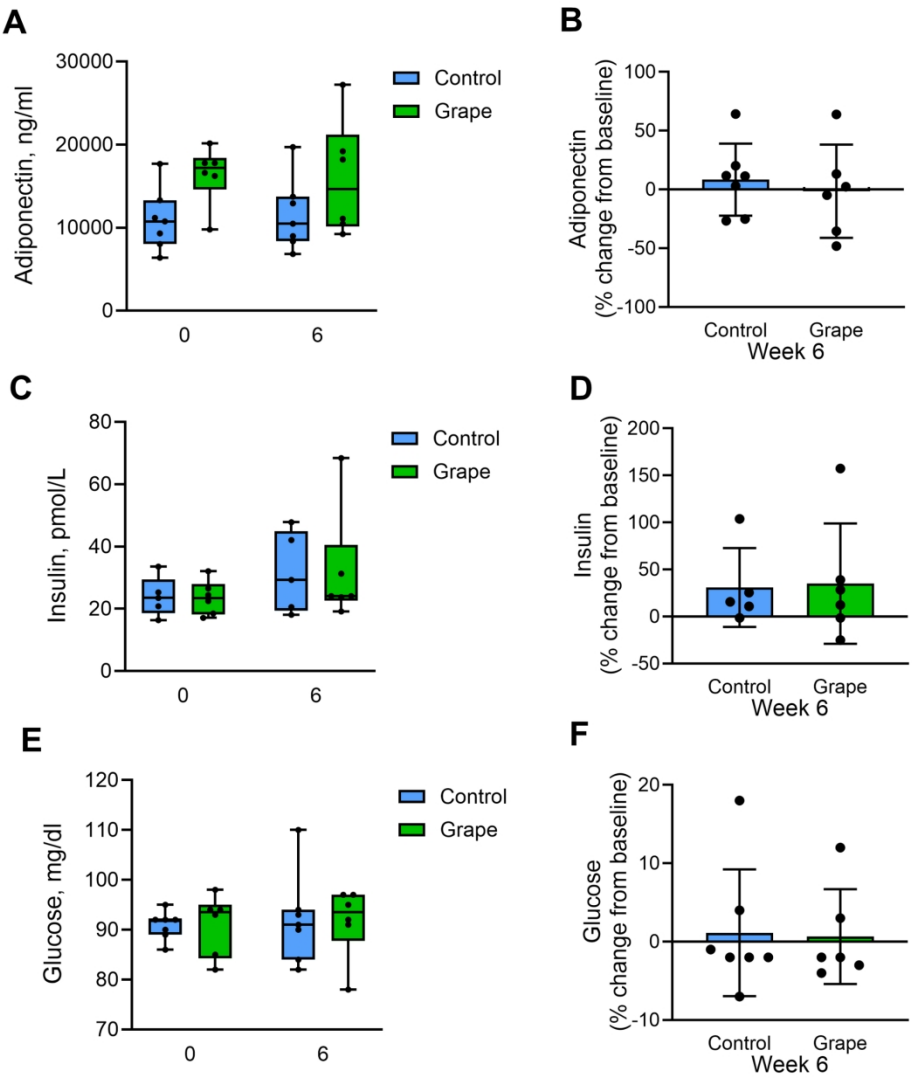

Figure 5

190x222mm (300 x 300 DPI)