The influence of daily grapes' intake on sarcopenia in postmenopausal women

Document date: 08/25/2022

1) Protocol Title

The influence of daily grapes' intake on sarcopenia in postmenopausal women

Protocol Version Date:

This is a new IRB submission

2) Objectives

The objective of this project is to evaluate whether the daily consumption of a freeze-dried table grape powder, rich in phenolic compounds, mitigates sarcopenia in postmenopausal women. To achieve this objective, we will pursue the following aims:

1. To determine the efficacy of a freeze-dried table grape powder, rich in phenolic compounds, to mitigate sarcopenia parameters in postmenopausal women.
1. To evaluate the effect of a freeze-dried table grape powder on key metabolic regulators of sarcopenia in postmenopausal women, specially irisin.

We hypothesize that daily dietary supplementation with a grape powder improves health span, mitigates sarcopenia parameters and increases irisin plasma levels in postmenopausal women.

The successful completion of this pilot human study will provide evidence on the benefit of the daily addition of table grape to the diet of healthy older women to improve sarcopenia by regulating some key metabolic regulators. This will provide strong preliminary results to apply for competitive grant (USDA) in order to expand these findings using a bigger sample size, multiple doses and various time-periods.

3) Background

As stated above, sarcopenia is defined as a loss of muscle strength and mass in older individuals. However, until recently, there has been no widely accepted definition of

sarcopenia that was suitable for use in research and clinical. Sarcopenia leads to fragility and disability, worsens the prognosis of many diseases, and significantly enhances morbidity and mortality. Furthermore, a higher risk of death from all causes compared with non-sarcopenic subjects was found in > 60 years old and mainly in > 80 years old (13, 14). With the increasing life expectancy and rapid growth of the aged population, sarcopenia is becoming an emerging public health issue with huge socioeconomic burden.

In recent years, various international consensuses have brought these fundamental concepts to the clinical practice. The European Working Group on Sarcopenia in Older People (EWGSOP) developed a practical clinical definition and consensus diagnostic criteria for age-related sarcopenia, recommending the use of the presence of both low muscle mass and low muscle function (strength or performance) for diagnosis. Recently, a new consensus (EWGSOP2) recommended low muscle strength as the primary parameter to assess sarcopenia and new cut-off points to increase harmonization of sarcopenia studies (15).

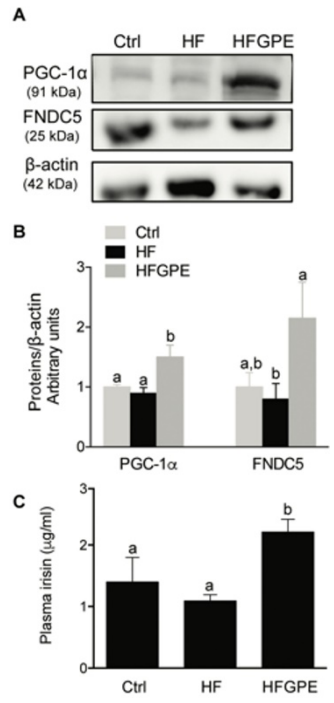

Figure 2: GPE supplementation activates the FNDC5/Irisin pathway in skeletal muscle from rats fed a High Fat diet. Rats were fed a control diet (Ctrl), or a High fat diet without (HF) or with supplementation with GPE (HFGPE) for 6 weeks. **(A)** Representative Western blot bands for PGC-1α and FNDC5 in total fractions isolated from skeletal muscle. **(B)** Bands were quantified, and results for PGC-1α and FNDC5 were normalized by β-actin levels (loading control). **(C)** Irisin plasma levels were determined by ELISA. Data are presented as means ± SEM of seven animals/treatment. Values having different superscripts are significantly different ($p < 0.05$, one-way ANOVA).

Given the above, there is an ongoing intensive search for novel therapies, including dietary ones that can attenuate the loss of muscle mass and strength in the elderly (10, 16). Interestingly, a growing body of research indicate that nutritional factors can preserve muscle function from age-related decline. In particular, phenolic compounds, widely distributed in fruits including grapes, have shown beneficial effects in muscle health (17, 18). Phenolic compounds have garnered attention because they ability to alleviate disease pathologies through anti-inflammatory, anti-carcinogenic and antioxidant

properties. However, the exact influence of grapes and grapes phenolic compounds mitigating sarcopenia in humans remains unexplored.

A novel and understudied mechanism by which skeletal muscle might mediate their protective effect against sarcopenia is by secreting myokines. Irisin is a myokine regulated by peroxisome proliferator-activated receptor gamma co-activator-1α (PGC-1α) and released into the bloodstream after cleavage of the Fibronectin type III domain-containing protein 5 (FNDC5) **(Fig 1.)**. Recent studies have indicated that circulating irisin can promote skeletal muscle growth (12, 19, 20). Lower irisin concentrations have been observed in sarcopenia patients (21) and dialyzed patients with cardiovascular disease (22). Furthermore, lower irisin plasma levels are associated with sarcopenia in postmenopausal women (10).

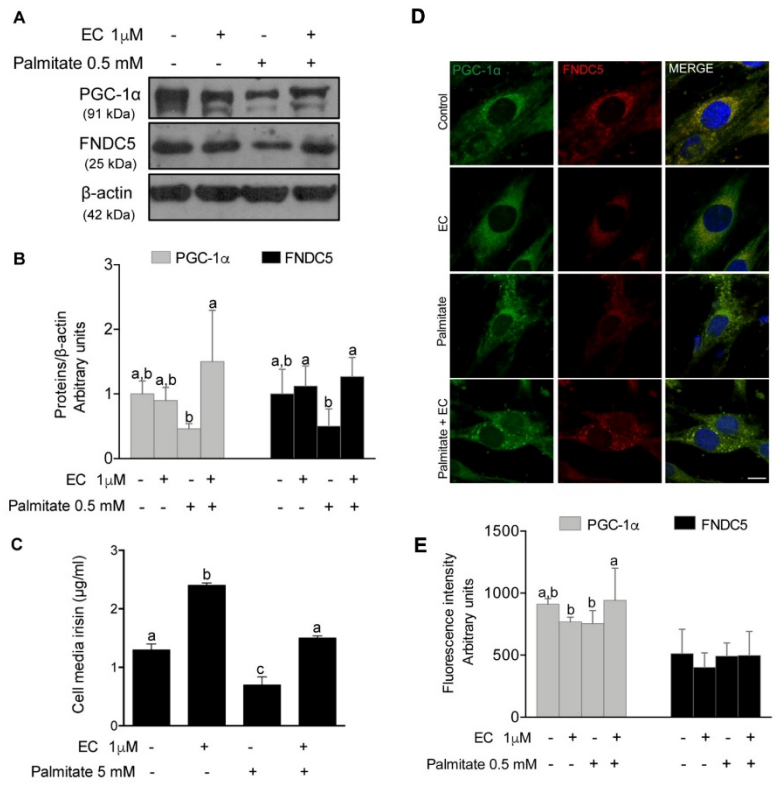

Figure 3: EC activates the FNDC5/Irisin pathway in L6 myotubes. L6 myotubes were incubated without or with 1 μM (-)-Epicatechin (EC) in the absence or presence of palmitate for 24 h. **(A)** Representative Western blot bands for PGC-1α and FNDC5. **(B)** Bands were quantified, and results for PGC-1α and FNDC5 were normalized by β-actin levels (loading control). **(C)** Irisin concentration in the cell culture media following treatment. **(D)** Representative immunofluorescent images for PGC-1α (*green*) and FNDC5 (*red*) in L6 myotubes. **(B)** Images were quantified, and results for PGC-1α and FNDC5 levels are displayed. For all experiments, data represent means ± SEM of three independent experiments. Values having different superscripts are significantly different ($p < 0.05$, one-way ANOVA).

In a recent study, we have shown that phenolic compounds present in a grape pomace extract (GPE), prevent irisin downregulation in rats fed a high-fat diet and in L6 myotube cells. In particular, we documented that consumption of the GPE activates the FNDC5/irisin pathway, with a significant increase of PGC-1α and FNDC5 protein

levels compared to control and high fat diet groups (**Figure 2A-B**). Furthermore, supplementation with GPE led to the increase in irisin plasma level (**Figure 2C**)(11).

In order to understand the underlying mechanism involved in the GPE-related irisin increased levels, we next evaluated the effects of epicatechin, one of the main flavonoids present in GPE, in L6 myotubes challenged with palmitate, to mimic a condition of fatty acids oversupply. Protein levels of PGC- 1α and FNDC5 were significantly higher in the EC and palmitate-treated cells, compared with palmitate-treated cells (**Figure 3A-B**). The same tendency was observed by immunocytochemical analysis of PGC-1α, while no differences were observed in FNDC5 fluorescence intensity among treatments (**Figure 3D-E**). In addition, EC induced a 2-fold increase in irisin levels secreted to the medium compared with cells treated with only palmitate (**Figure 3C**). These data strongly indicate that phenolic compounds enhance irisin levels in vitro and in vivo.

These intriguing data allow us to hypothesize that phenolic compounds present in grapes might have a positive effect in human health, specially preventing sarcopenia in elderly humans, in part, by increasing irisin levels.

4) Enrollment Numbers

We chose the "changes in plasma irisin levels" as our primary outcome to determine the power calculations for sample size. Based on our preliminary data (11), 5 individuals per group are required to reach 80% power to detect differences in irisin levels between the groups, at a α=0.05, using a two-sided paired T-test. We propose to enroll 7 individuals per group, instead of 5, in the event of a patient drop out of the study (for any unforeseen circumstances). In total we will have 14 subjects.

5) Inclusion and Exclusion Criteria

Inclusion Criteria: postmenopausal women aged 60 years or older with a hand-grip strength media value of 16 kg or lower, normal body mass index (BMI) with values between 18.4 and 24.9 kg/m2, blood pressure below 130/80 mmHg, and plasma glucose and cholesterol concentrations below 100 and 200 mg/dl.

Exclusion Criteria: history of 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, on dialysis or long-term steroid therapy, and/or actively receiving treatment for cancer or severe infection (excluding short-term antibiotic therapy), weight less than 140 pounds, anemia and blood donations in the past 30 days. In addition, participants should not be taking dietary supplements at the time of the study.

6) Recruitment Methods

We will recruit by: 1) posting flyers on campus bulletin boards; 2) listing the study on our departmental website that provides a list of all current departmental trials; 3) listing the study on campus list servers; 4) advertising in the local newspapers and 5) posting on social media. All recruitment material will use similar language and list a study office phone number, clinical coordinator email and the departmental website for clinical trials (**Attachment A**). Respondents will be contacted by phone and pre-screened. Information about the study will be provided during the phone interview (**Attachment B)**. For subjects that are eligible and interested in enrolling in the study, an in-person screening visit 1 appointment will be scheduled with the investigator or designee and the informed consent form emailed to them in advance. If the participants meet all the inclusion criteria, in the same visit the investigator or designee will review the informed consent form with the subjects, explain the study to the subjects and answer any questions prior signing the consent. Only once the informed consent form is signed, a diet history questionnaire (**Attachment C**) will be completed by the subjects.

7) Compensation to the Subjects

Subjects will be compensated at a rate roughly proportional to the inconvenience

incurred according to the following schedule:

- Visit 0 (screening): $50 for inconvenience
- Visit 1: $75 for inconvenience
- Visit 2: $75 for inconvenience
- Visit 3: $75 for inconvenience + $50 for study completion

Subjects will be paid at the completion of their involvement in the study. The total compensation that a participant might earn is $325 for completion of all visits. Subjects that withdraw from the study early will be compensated based on the visit(s) they completed.

8) Screening procedures to determine eligibility:

After being pre-screened for eligibility by a phone interview, eligible potential participants will come to the Ragle Facility in a fasting (12 h) state for the in-person screening **visit 0**. In this visit the participants will sign an informed consent form. The subject will read the information carefully and will be given the opportunity to seek more information if needed. The investigator or designee will obtain written (signed and dated by the subject) informed consent from each potential participant after adequate explanation of the aims, methods and potential hazards of the study. The investigator or designee will also explain to the potential participant that his/her participation is voluntary, and he/she is free to withdraw from the study at any time. The investigator or designee will sign and date the consent form to confirm that the consent process was completed correctly. The subject will also be provided with the option of taking the consent form home to review prior to making his/her decision. The subject will be provided with a copy of his/her signed and dated consent form. Once consent has been obtained, the pre-screening visit will proceed. After the subject has signed the informed consent, an identification number (ID) will be assigned and entered the screening and enrollment log. A diet history questionnaire **(Attachment C)** will be complete by the participant.
After this we will confirm if they meet the following inclusion criteria:

1- Hand-grip strength media value of 16 kg or lower: Handgrip strength will be evaluated by using a digital dynamometer (Grip Strength Dynamometer T.K.K.5401; Takei Co., Tokyo, Japan). Following are the steps of the procedure [a detailed procedure will be added (**Attachment D**)]:
   - The participant will be asked to seat comfortably in a standard chair with legs, back support and fixed arms.
   - The participant will be asked to rest their forearms on the arms of the chair with their wrist just over the end of the arm of the chair wrist in a neutral position, thumb facing upwards.
   - The person in charge of the test will demonstrate how to use the handgrip dynamometer.
   - The participant will be asked to start with the right hand.
   - The person in charge of the test will encourage the participant to squeeze as long and as tightly as possible or until the needle stops rising. Once the needle stops rising the participant can be instructed to stop squeezing.
   - The person in charge of the test will read grip strength in kilograms from the outside dial and record the result to the nearest 1 kg on the data entry form.
   - The person in charge of the test will ask the participant to repeat measurement in the left hand.
   - The participant will be asked to repeat two further measurements for each hand alternating sides to give three readings in total for each side.
   - The best of the 3 grip strength measurements will be use in statistical analyses to encourage the subjects to get as high a score as possible.
   - The person in charge will also record hand dominance, i.e. right, left or ambidextrous (people who can genuinely write with both hands).

For those participants that meet the previous inclusion criteria the following parameters will be explore:

1. Seated resting blood pressure.
2. Weight and height.

3. A finger stick blood sample (~1-2 drop equal to ~ 100ul) will be collected to confirm that subjects meet the inclusion criteria for fasting levels of blood glucose and cholesterol, plus a hematocrit to discard anemia.

Briefly, at Visit 0 (screening visit):

- Subjects will arrive fasted for at least 12 h prior to this visit (water ok).
- Informed consent will be obtained.
- ID number will be assigned.
- Hand grip strength test will be conduct.
- Seated resting blood pressure will be measured.
- Weight and height will be measured.
- A finger stick blood sample (~1-2 drop equal to ~ 100ul) to confirm that subjects meet the inclusion criteria (glucose, cholesterols and hematocrit).
- Diet history questionnaire (**Attachment C**) will be completed by the subjects to review medical history (including known positivity test for COVID-19), concomitant medications (including COVID-19 immunization dates), inclusion and exclusion criteria.
- This visit will take approximately 2 h to complete

9) Procedures Involved

Schedule of procedures

	Visit 0 Screening	**Visit 1 Baseline**	**Visit 2 3-weeks visit**	**Visit 3 6-weeks final visit**
Informed consent	X			
Inclusion / Exclusion criteria	X			
Review medical history	X			
Review concomitant medications	X		X	
Height, weight and blood pressure	X	X	X	X
Finger stick		X	X	X
Hand grip strength test	X	X	X	X
SPPB test		X	X	X

Blood draws for plasma determinations	X	X	X	X
Hematocrit	X	X	X	X
Study product randomization		X		
Study product intake			X	X
Dispense study product for home		X	X	
Return unused study product and empty packages			X	X
Dispense stool and urine collection kit		X	X	
Return stool and urine collection kit			X	X
Adverse events assessment			X	X

After confirmation of the inclusion/exclusion criteria enrolled subjects will be asked to travel to Ragle Human Nutrition Center at University of California Davis for 3 study visits: a baseline visit (**visit 1**), and a 3-week intervention visit (**visit 2**) and a final 6-week visit (**visit 3**)

Visit 1 (baseline visit):

On this visit, baseline measurement will be done. Fasted participants (12 h) will provide blood samples and complete two tests:

Blood samples**:** from each participant 20 ml of blood will be extracted. The following parameters will be measured in plasma, (the same parameters will be measure in the following visits 2 and 3):

Blood parameter	Volume of blood needed	Visit 1	Visit 2	Visit 3
Irisin	1200 ul	x	x	x
Glucose	1000 ul	x	x	x
Insulin	1200 ul	x	x	x
GLP-1/ 2, GIP	2000 ul	x	x	x
Adiponectin, leptin and ghrelin	3000 ul	x	x	x
Blood urea nitrogen, creatinine	1000 ul	x	x	x
Total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein	2000 ul	x	x	x
Aspartate transaminase, Alanine transaminase	2000 ul	x	x	x
IL-6, IL-15	2000 ul	x	x	x
25-hydroxyvitamin D	1000 ul	x	x	x
Hematocrit	500 ul	x	x	x

Total volume of blood per visit	~ 20 ml	20 ml	20 ml	20 ml

After taking the blood samples a snack will be provide to the participants in order to complete the following tests.

1. Hand-grip strength: previously describe in the screening point
2. Short Physical Performance Battery (SPPB)**:** the SPPB score will be based on three tests, as follows: balance test, gait speed test, and chair stand test (see **Attachment E**). Of note, these tests have been used as a predictive tool for possible disability and can aid in the monitoring of function in older people.

Following, the subjects will randomize into the study supplement: a) Placebo powder (46 g placebo powder in 180 ml of water); or b) freeze-dried table grape powder (46 g grape powder in 180 ml of water). The supplement will be supplied to the participants to complete the following 3 weeks supplementation until the next visit. The investigator or designee will explain the details regarding the preparation and consumption of the supplement [a dosing protocol will be uploaded too (**Attachment F**)].

Also a 24-hs food and physical activity questionary will be performed

Briefly, at visit 1:

- Subjects will arrive fasted for at least 12 h prior to this visit (water ok).
- A finger stick blood sample will be collected (to confirm fasting).
- Concomitant medications and accomplishing of inclusion and exclusion criteria will be reviewed.
- 24-hs food and physical activity questionary
- Seated resting blood will be measured.
- Weight will be measured.
- Venous blood samples will be collected for analytical determinations (20 ml).
- A hematocrit will be performed in order to discard anemia.
- Snack ingestion
- Hand grip strength and SPPB test will be completed.

- The supplement will be provided to the subjects for a 3 weeks supplementation period.
- Stool and urine collection kit and a standardized log and instructions (**Attachment G and H**) will be provided to subjects. The kits will be returned at visit 2.

Visit 2 (3-weeks visit):

Subjects will return to the Ragle Facility for the follow-up visit. This visit will take approximately 2 h to complete.

Briefly, at visit 2:

- Subjects will arrive fasted for at least 12 h prior to this visit (water ok).
- Subjects will provide fecal and urine samples collected with the collection kit.
- A finger stick blood sample will be collected (~1 drop).
- Concomitant medications and accomplishing of inclusion and exclusion criteria will be reviewed.
- 24-hs food and physical activity questionary
- Seated resting blood pressure will be measured.
- Weight will be measured.
- Subjects will take the assigned supplement.
- Venous blood samples will be collected (20 ml).
- A hematocrit will be performed in order to discard anemia.
- Snack will be provided to the participants.
- Hand grip strength and SPPB test will be completed.
- Enough supplement will be provided to the subjects for a 3 weeks supplementation period.
- Unused and empty packages of the study product will be return.
- Stool and urine collection kit will be provided to subjects. The kits will be returned at visit 3.

Visit 3 (final visit):

Subjects will return to the Ragle Facility for the final visit. This visit will also take approximately 2 h to complete.

Briefly, at visit 3:

- Subjects will arrive fasted for at least 12 h prior to this visit (water ok).
- Subjects will provide fecal and urine samples collected with the collection kit.
- A finger stick blood sample will be collected (~1 drop).
- Concomitant medications and accomplishing of inclusion and exclusion criteria will be reviewed.
- 24-hs food and physical activity questionary
- Seated resting blood pressure will be measured.
- Weight will be measured.
- Subjects will take the assigned supplement.
- Venous blood samples will be collected (20 ml).
- A hematocrit will be performed in order to discard anemia.
- Snack will be provided to the participants.
- Hand grip strength and SPPB test will be completed.
- Unused and empty packages of the study product will be return.

10) Study Timelines

Duration of individual subject's participation will be ~6 weeks. The study will include: 1 visit (visit 0) for the screening process that will last approximately 2 h and 3 more visits (visits 1, 2 and 3) for intervention process that will last approximately 2 h each. Visit 0 will involve an in-person screening to obtain written informed consent and determine if subjects meet the inclusion/exclusion criteria. Visit 1, 2 and 3 (at 0, 3 weeks and at the end of the 6 weeks, respectively) will include blood sample collection and tests performance.

11) Study Endpoints

Primary endpoint:
Hand-grip strength media value of 16 kg or lower

Secondary endpoints:
Levels of Irisin as a marker of sarcopenia
Glucose, Insulin, GLP-1, GLP-2, GIP, Adiponectin, leptin and ghrelin, Blood urea nitrogen, creatinine, Aspartate transaminase, Alanine transaminase, Total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein, IL-6, IL-15, 25-hydroxyvitamin D as markers of general metabolism state.

12) Data and/or Specimen Management and Confidentiality

Data management: The de-identified data will be analyzed for statistical significance with the help of the Biostatistics group at UC Davis, including univariate graphical, analytic summaries and bivariate relationships using box plots, scatter plots, and correlations. To compare participants in the two diet groups (Placebo control and freeze-dried table grape powder) a paired Student's t test (two-tailed) will be used. Data involving more than two repeated measures, such as grip strength tasks, or irisin levels, will be assessed by repeated measures (ANOVA). Differences will be considered significant at $P < 0.05$. Significant results will be also subjected to analysis of covariance (ANCOVA) to account for group differences at baseline and post hoc comparisons using the Bonferroni correction.

Data confidentiality: After obtaining written informed consent, all subjects will be assigned an identification number (ID) and all data and specimens will be collected from individuals and stored using this ID number. The key linking the subjects' personal information to their laboratory results will be kept in a locked drawer in the investigator's office. Only authorized research personnel will have access to the study data. People outside the research process will not have access to results about any one person. Staff analyzing the data will only have access samples identified by subjects' ID number. The subjects'

personal information (questionnaires and consent form) will be kept separately under lock and key in the investigator's office

13) Withdrawal of Subjects

Possible reasons for removal include 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, on dialysis or long-term steroid therapy, and/or actively receiving treatment for cancer or severe infection (excluding short-term antibiotic therapy) that are diagnosed ones the subject are already enrolled in the study. If the participants start taking supplements. Also, if the participants experiment a remarkable increment in body weight, blood pressure and plasma glucose and cholesterol concentrations above 100 and 200 mg/dl. Finally, development of anemia during the study will be another reason for removal.

14) Risks to Subjects

Venipuncture: Risks of venipuncture include some discomfort, bruising, and rarely, infection. Blood collections will be performed by a registered nurse to minimize discomfort.

Blood volume: The amount of blood to be drawn during the study will be ~60 mL, collected over a period of 6-weeks. We will take a finger stick blood sample (~1 drop, equal to ~100 ul) at visits 0,1, 2 and 3 and 20 mL/visit at visits 1, 2 and 3. These volumes are well within the recommended allocated volume for healthy adults. Nevertheless, the patients could experiment dizziness, nausea and physical weakness.

Study products: The components of the dietary supplement and placebo are detailed in **Attachment I** and **Attachment J,** respectively. The dose that the participant will consume daily is the equivalent to a ~150 g (1.5 cups) of fresh grapes, which won't represent a health risk for the participant

15) Potential Benefits to Subjects

No direct benefits to the individual subjects are anticipated. However, subjects will receive general information regarding their overall health status.

16) Provisions to Protect the Privacy Interests of Subjects

Subjects will be assigned an identification number (ID) and all data and specimens will be collected and stored using this ID number. Personal information (name, phone number and email address) and consent forms will be kept in confidence and will be stored in a locked cabinet in the investigator's office. The key linking the subjects' personal information to their laboratory results will be kept separately in another locked drawer in the investigator's office (See above for more on confidentiality).

At the Ragle Facility, the subjects will interact with the study coordinator and trained research personnel. The data collection (anthropometry, blood pressure and venipuncture) will be performed in individual secluded rooms by trained research personnel. We will ensure that the subjects feel comfortable with all research procedures. Questions and interactions between the subjects and the research team will be kept private.

17) Sharing of Results with Subjects

Subjects will receive the results of their blood screen (once reviewed and approved) at the time of completion of the study (or early withdrawal). The research data will not be shared with the subjects, but a summary of the research can be provided to the subjects who express interest.

18) Data/Specimen Banking

All samples, including those sent out for outside analysis will have only a numerical identifier and date/time. Specimens to be stored on site will be kept frozen at -80°C in secure laboratories. Specimens will be stored as long as they are viable for analyses; records of storage will be kept by the investigator's lab staff; all records will be of the de-identified specimens. During analyses, the de-identified data will be entered in Excel

files and analyzed for statistical significance using SAS in password protected computers.

19) Multi-Site Research

This is a single site study.

20) Community-Based Participatory Research

N/A

21) Review Requirements

Some research projects require specific IRB determinations.

1. Are there any contractual obligations or other considerations that require IRB review of this research, or review at intervals other than those required by the Common Rule or FDA?

   ☐ Yes

   ☒ No

Title of research study: The influence of daily grapes intake on sarcopenia in postmenopausal women

Investigator: *Prof. Gerardo G. Mackenzie*

Why am I being invited to take part in a research study?

We invite you to take part in this research study because we are looking for healthy postmenopausal women aged 60 years or older in order to study the effects of grapes on muscle health.

What are my rights as a research subject?

(Experimental Subject's Bill of Rights)

Someone will explain this research study to you, including:

1. The nature and purpose of the research study.
2. The procedures to be followed.
3. Supplement to be used.
4. Any common or important discomforts and risks.
5. Any benefits you might expect.
6. Other procedures and their risks and benefits compared to this study.
7. Whether or not you take part is up to you.
8. You can choose without force, fraud, deceit, duress, coercion, or undue influence.
9. You can choose not to take part.
10. You can agree to take part now and later change your mind.
11. Whatever you decide it will not be held against you.
12. You can ask all the questions you want before you decide.
13. If you agree to take part, you will be given a signed and dated copy of this document.

How is this research funded?

This research is being funded by California Table Grape Commission.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, you may talk to the research director: Prof. Gerardo Mackenzie 530-752-2140

This research has been reviewed by an Institutional Review Board (IRB). Information to help you understand research is on-line at https://research.ucdavis.edu/policiescompliance/irb- admin. You may talk to a IRB staff member by phone: (916) 703-9158, by email: hs-irbeducation@ucdavis.edu, or by mail: 2921 Stockton Blvd, Suite 1400, Room 1429, Sacramento, CA 95817 for any of the following:

Your questions, concerns, or complaints are not being answered by the research team.
You cannot reach the research team.
You want to talk to someone other than the research team.
You have questions about your rights as a research subject.
You want to get information or give input about this research.

Why is this research being done?

The purpose of the study is to assess the consumption of freeze-dried table grape powder on the muscle strength of post-menopausal women.

How long will the research last?

We expect that you will be in this research study for about 6 weeks. During the 6 weeks you will be asked to consume daily a beverage containing either: (A) Placebo powder (46 g placebo powder in 180 ml of water, equal to about 6 ounces); or (B) freeze-dried table grape powder (46 g freeze-dried table grape powder in 180 ml of water, equal to about 6 ounces). In addition, we will ask you to come to 4 visits of about 2 hours each: a screening visit (visit # 0), a baseline visit

(visit # 1) a mid-term visit (3 weeks, visit # 2: data and sample collection) and a final visit (6 weeks, visit # 3: data and sample collection)

How many people will be studied?

We expect about 14 people to complete the research study.

What happens if I say yes, I want to be in this research?

If you decide to participate in this research study, you will be asked to complete this visit (visit 0) and 3 extra study visits scheduled as follows:

Visit 0	Visit 1	Visit 2	Visit 3
Today Screening visit	Baseline visit Week 0	Follow up visit Week 3	Final visit Week 6

Below, we describe the procedures and commitments that are required for this study:

<u>At visit 0 (screening visit):</u>

- You will be asked to stop consuming food or beverages for at least 12 hours the day before of this visit (we have explained this to you in the telephone screening). During this fasting period, you will be able to drink as much water as you need.
- You will arrive to the Ragle Facility fasted.
- You will remain at the Ragle Facility for the duration of this visit (about 2 hours).
- You will read and sign the informed consent.
- You will perform a hand grip strength test (explained below).
- Resting blood pressure will be measured.
- Weight and height will be measured.
- A finger stick blood sample will be taken (about 1-2 drops) to confirm if you meet the

inclusion criteria for fasting levels of blood glucose, cholesterol, and hematocrit.

- You will complete a diet history questionnaire, concomitant medications (including COVID-19 immunization dates), inclusion and exclusion criteria.

At visit 1 (baseline visit):

- You will not consume any food or beverages for at least 12 hours the day before your visit. During this fasting period, you may drink as much water as you wish.
- You will arrive to the Ragle Facility fasted.
- You will remain at the Ragle Facility for the duration of this visit (about 2 hours).
- A finger stick blood sample (about 1 drop) will be collected upon arrival to confirm you are fasting. If you are not sufficiently fasted, you will be asked to reschedule the visit for a future day.
- We will check the medications you take and whether you meet the inclusion and exclusion criteria.
- We will perform a 24-hs food and exercise questionary.
- Blood pressure will be measured.
- Weight will be measured.
- A blood sample will be collected (20 ml, equal to about 1.5 tablespoon) in order to measure the parameters that we will study.
- A snack will be provided to you.
- Hand grip strength and Short Physical Performance Battery (SPPB) test will be completed (both explained below).
- We will provide you with the study product to consume for the next 3 weeks.
- You also will receive a stool and urine collection kit and instructions in order to collect samples that you will bring to the next visit. You will be asked to collect a stool sample within 16 hours before visit 2 and to bring the container with sample on the day of visit. For urine collection you will fill a 120 ml cup (we will provide the cup and instructions) with the first urine of the morning from the same day of visit 2. Both samples can be

kept in the fridge until the visit. We will repeat this at the following visit, so in total you will provide 1 sample of urine and 1 sample of stool twice during the protocol.

At visit 2 (Follow-up visit week 3):

- You will not consume food or beverages for at least 12 hours the day before your visit. During this fasting period, you may drink as much water as you wish.
- You will arrive to the Ragle Facility fasted.
- You will remain at the Ragle Facility for the duration of this visit (about 2 hours).
- A finger stick blood sample (about 1 drop) will be collected upon arrival to confirm you are fasting. If you are not sufficiently fasted, you will be asked to reschedule the visit for a future day.
- You will provide the fecal and urine samples that you collected the night before and that morning, respectively.
- We will check the medications you take and whether you meet the inclusion and exclusion criteria.
- We will perform a 24-hs food and exercise questionary.
- Blood pressure will be measured.
- Weight will be measured.
- A blood sample will be collected (20 ml, equal to about 1.5 tablespoon).
- A snack will be provided to you.
- We will measure your hand grip strength and an SPPB test will be completed.
- We will provide you with more study product for the final 3 weeks.
- You will return the empty packages of study product consumed in the last 3 weeks.
- We will provide you with new stool and urine collection kits and you will be asked to bring the samples to the next visit.

At visit 3 (Final visit week 6):

- You will not consume any food or beverages for at least 12 hours the day before your visit. During this fasting period, you may drink as much water as you wish.You will arrive to the Ragle Facility fasted.
- You will remain at the Ragle Facility for the duration of this visit (about 2 hours).
- A finger stick blood sample (about 1 drop) will be collected upon arrival to confirm you are fasting. If you are not sufficiently fasted, you will be asked to reschedule the visit for a future day.
- You will provide fecal and urine samples that you collected the night before and that morning, respectively.
- We will check your medications and whether you meet the inclusion and exclusion criteria.
- We will perform a 24-hs food and exercise questionary.
- Blood pressure will be measured.
- Weight will be measured.
- Blood samples will be collected (20 ml, equal to about 1.5 tablespoon).
- A snack will be provided to you.
- We will measure your hand grip strength and an SPPB test will be completed.
- You will return the unused study product and the empty packages of study product consumed in the last 3 weeks.

Starting from visit 1 and throughout the study, we will also ask you to limit the consumption of some foods that contain high concentrations of polyphenols. We will ask you to limit your consumption of blueberries, blackberries, bilberries, cherries, grapes, grape juice, pomegranate, raspberries, huckleberries, strawberries to less than ½ cup per day. We will also ask you to drink less than 1 glass of red wine, and less than 3 cups of tea or coffee, and to consume less than 10 g (about 0.35 ounces) of chocolate per day.

On each visit you will be asked to perform a handgrip strength test as follows: you will be asked

to sit comfortably in a standard chair with legs, back support, and fixed arms. An assistant will show you how to use the handgrip dynamometer. You will be encouraged to squeeze the dynamometer as long and as tightly as possible or until the needle stops rising. We will measure your hand strength in both hands 3 times. At visits 1, 2 and 3 you will also be asked to perform an SPPB test, which is based on three easy tests: (1) A balance test: you will be asked first to stand with your feet together, side-by-side, for about 10 seconds, then to stand with the side of the heel of one foot touching the big toe of the other foot for about 10 seconds, and finally to stand with the heel of one foot in front of and touching the toes of the other foot for about 10 seconds. (2) A gait speed test: you will be asked to twice walk to the other end of the course at your usual speed, just as if you were walking down the street to go to the store. (3) A chair stand test: you will be asked to stand up from a chair without using your arms 6 times.

For the study visits you will go to the Ragle Human Nutrition Center at University of California Davis. During the visit 0 screening process, the investigator or designee will read and explain to you all the details of the study. You may ask all the questions you may have about the study. If you agree with all the terms and have received answers to all your questions, you will be asked to sign the informed consent form. An identification number (ID) will be assigned for you. You will be asked to complete a diet history questionnaire. We will then measure your resting blood pressure, weight and height, and a blood sample will be collected to confirm that you meet the inclusion criteria for fasting levels of blood glucose, cholesterol, and hematocrit. Once you are enrolled in the study, you will be randomly assigned to two different supplement groups, consuming either a **placebo powder** (46 g placebo powder); or **a freeze-dried table grape powder** (46 g grape powder). The group will be chosen by chance, like flipping a coin. Neither you nor the study investigator will choose what supplement you will get. You will have an equal chance of being in either group. The study product will be provided to you during visits 1 and 2 and you will be asked to consume it every day for 6 weeks by dissolving it in 180 ml of water (about 6 ounces). We will provide you with the supplement in powdered form in an appropriate container and you will be asked to reconstitute the powder with water in a glass/container and then mix it and consume it within 30 min from reconstitution.

What are my responsibilities if I take part in this research?

If you take part in this research, you will be responsible to:

1. Follow the procedures in preparation for the study visits.
2. Participate in 4 study visits of about 2 hours each.
3. Complete a health and habits questionnaire.
4. Take the study supplement daily.
5. Provide blood samples: 20 ml, equal to about 1.5 tablespoon, at visits 1, 2 and 3.
6. Provide a finger stick blood sample (about 1 drop) at visits 0, 1, 2 and 3.
7. Perform the hand grip test at visits 0,1, 2 and 3 and the SPPB test at visits1, 2 and 3.
8. Perform the stool and urine sample collection.
9. Follow the diet restrictions that we detailed for you.

What happens if I do not want to be in this research?

You may decide not to take part in the research and it will not be held against you.

What happens if I say yes, but I change my mind later?

You can leave the research at any time and it will not be held against you.

If you decide to leave the research, contact the investigator so that the investigator can be aware of that.

If you stop being in the research, already collected data may not be removed from the study database.

Is there any way being in this study could be bad for me?

This is a minimal risk research study. That means that the risks of participating are no more likely or serious than those you encounter in everyday activities. The possible risks or discomforts include:

1. Blood drawing can result in bruising, mild discomfort, and rarely, an infection where the needle enters the skin. There is also a possibility of dizziness, nausea, and physical weakness.
2. The supplement is a lyophilizate powder made with fresh grapes. You could experience some minor intestinal discomfort related to ingestion of the grape powder.
3. If you have food allergies associated with any ingredients in the study products, you should inform the study staff so that your health is not put at risk. Food allergies can be dangerous. You should not participate in this study if you have specific food allergies associated with the components in the study supplement.
4. Loss of confidentiality is a potential risk; however, every measure will be taken to ensure that your protected health information is kept safe. Study records are kept behind locked doors and on secure servers to which only a limited number of trained study staff members have access.

Will being in this study help me in any way?

We cannot promise any benefits to you from your taking part in this research.

What happens to the information collected for the research?

Efforts will be made to limit use or disclosure of your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete confidentiality. Organizations that may inspect and copy your information include the IRB, the California Table Grape Commission, and other University of California representatives responsible for the management or oversight of this study

If you agree to participate in this research study, a signed copy of this consent document and the privacy authorization form may be filed in your electronic medical record (EMR) and your study participation may be added to your EMR. This information will be used for your care and for healthcare operations, which may include billing and payment. Federal and state privacy laws give patients the right to access information about their care and study contained in their medical record. During this study, you may not be able to access certain information related to this study

in your EMR until the study is complete to ensure that the study remains unbiased. By consenting to participate in this study, you are also consenting to this possible temporary withholding of your research records.

The sponsor, monitors, and auditors will be given direct access to your research records to run and oversee the study. We may publish the results of this research. However, we will keep your name and other identifying information confidential. In addition, the IRB and the Food and Drug Administration (FDA) will be given direct access to your research records to ensure proper oversight of the study.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Can I be removed from the research without my OK?

The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include: 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, on dialysis or long-term steroid therapy, and/or actively receiving treatment for cancer or severe infection (excluding short-term antibiotic therapy) that are diagnosed after the subject is already enrolled in the study. You may also be removed if you start taking supplements after the start of the study, if you develop anemia, if you experience a remarkable change in body weight, blood pressure, glucose or cholesterol plasma levels, or you make any blood donations within 30 days before the start of the study.

What else do I need to know?

There is no charge for you to take part in this study. Neither you nor your insurance carrier will be charged for your taking part in the research. All costs associated with the study will be paid by the sponsor/department.

If you are injured as a result of being in this study, the University of California will provide the necessary medical treatment. Depending on the circumstances, the costs of the treatment may be covered by the University or the study sponsor or may be billed to your insurance company just like other medical costs.

Due to the coronavirus public health crisis, the federal government issued a Declaration under the Public Readiness and Emergency Preparedness (PREP) Act. If the Declaration applies, it limits your right to sue and recover for losses from the researchers, healthcare providers, any study sponsor or manufacturer or distributor involved with the study, including the University of California, while participating in this clinical study. However, the federal government has a program that may provide compensation to you or your family for certain claims if you experience serious physical injuries or death and these costs are not covered by other payors. To find out more about this "Countermeasures Injury Compensation Program" go to https://www.hrsa.gov/cicp/about/index.html or call 1-855-266-2427.

Will I be paid or receive anything for being in this study?

We will pay you a total $325 if you complete the full study. If you leave the study early, we will pay you the following prorated amounts for each visit of the study you complete:

- Visit 0 (screening): $50
- Visit 1: $75
- Visit 2: $75
- Visit 3: $75 + $50 for study completion

You may be asked for your social security number for payment purposes. It will not be used for any other reason without your permission.

If you receive $600 or more during a calendar year from the University for taking part in research, you may receive a 1099 for tax reporting purposes. Reimbursements for travel and other expenses are not included in this amount.

Biospecimens (such as blood, urine, and stool) collected from you for this study and/or information obtained from your biospecimens may be used in this research or other research and shared with other organizations. You will not share in any commercial value or profit derived from the use of your biospecimens and/or information obtained from them.

Are there other research opportunities?

If you are interested in being contacted for future research, please write your phone number and/or email. This is completely optional.

_____(initials) Yes, I am willing to be contacted for future research studies. My phone number and/or email is: _____.

Signature Block for Capable Adult

Your signature documents your permission to take part in this research.

Signature of subject	Date
Printed name of subject	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

Assigned participant ID#:_____