The Effect of Vitamin C-enriched Collagen Combined with Resistance Training on Muscle-tendon Unit Properties in Middle-aged Men and Women

Study protocol, statistical analysis plan and informed consent form.

Document date: 04/22/2024

1. Participant Flow

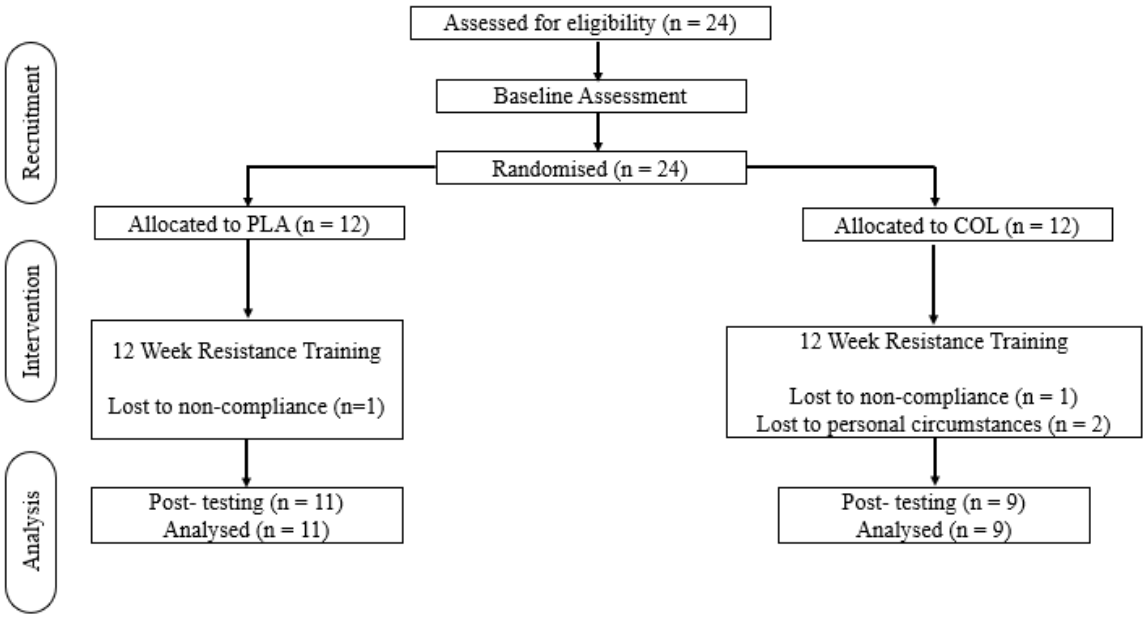

Figure 1. Flowchart of participant recruitment and intervention timeline (males).

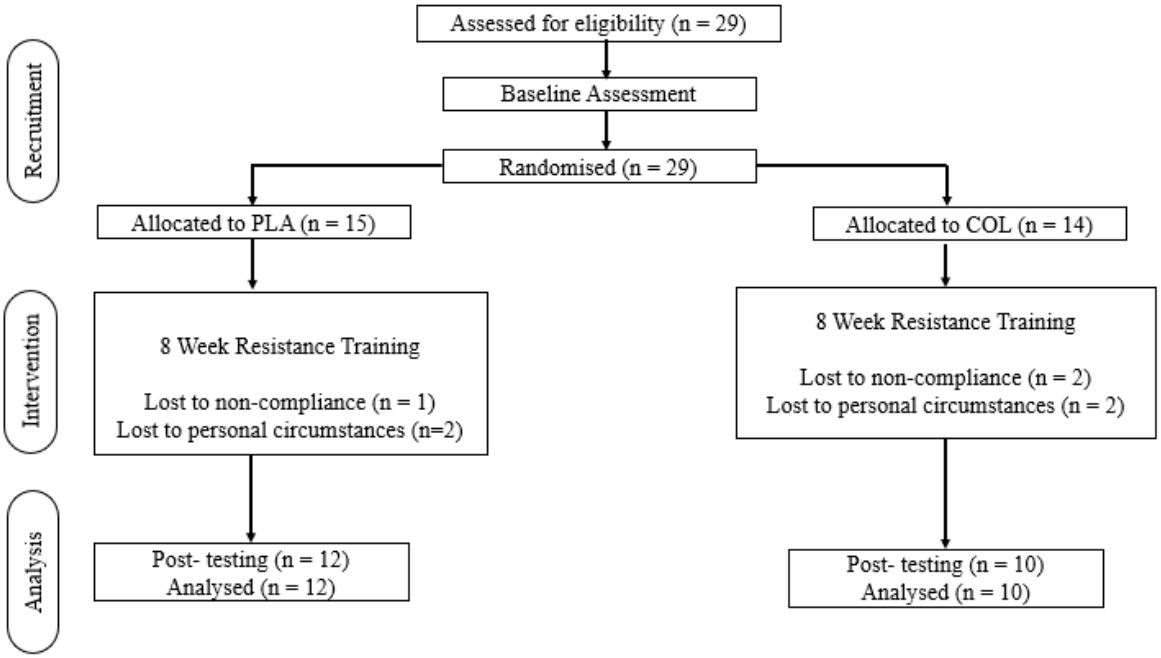

Figure 2. Flowchart of participant recruitment and intervention timeline (females).

Study Protocol

Brief Summary:

The aim of this clinical trial is to investigate the longitudinal effects of hydrolyzed collagen ingestion combined with resistance training on muscle-tendon unit structure and function in middle-aged males and females.

The main research questions this clinical trial aims to answer are:

1. Does resistance training with hydrolyzed collagen ingestion lead to greater changes in tendon properties than resistance training alone in middle-aged health men and women?

2. Does resistance training with hydrolyzed collagen ingestion lead to greater changes in muscle size than resistance training alone?

3. Does resistance training with hydrolyzed collagen lead to greater increases in strength and power compared to resistance training alone in middle-aged, healthy men and women?

Participants will be randomly assigned to collagen or placebo groups. Participants will perform 24 sessions of high intensity resistance training across 12-weeks. Alongside each training session, participants will consume a beverage containing hydrolyzed collagen or maltodextrin, with both beverages containing vitamin C.

Researchers will compare the collagen and placebo groups to see if there would be beneficial effects on changes in muscle and tendon that are greater than resistance training alone. To achieve this, an dynamometry will be used to assess lower limb strength and ultrasound will be used to measure the morphological, mechanical, and material properties of the patellar tendon, as well the size and architecture of the vastus lateralis muscle.

Detailed Description:

Healthy, active, middle-aged men and women will ingest a beverage containing 30 g of hydrolyzed collagen with 50 mg of vitamin C or a calorie matched beverage (maltodextrin), also with 50 mg of vitamin C combined with high-volume, high-intensity resistance training for the lower body on 2 - 3 occasions per week for 8-12 weeks.

The aim of this study was to investigate the effect of combining hydrolyzed collagen with resistance training in middle-aged men and women.If supplementation with hydrolyzed collagen leads to a greater change in tendon size, stiffness, and Young's modulus than resistance training alone, this will allow us to recommend this type of intervention to young athletes seeking to improve tendon health and/or athletic performance. This is the first study to investigate the combination of hydrolyzed collage supplementation with resistance training in middle-aged men and women. If supplementation with hydrolyzed leads to greater improvements in tendon size, stiffness, strength and/or power, this

will allow us to recommend this type of intervention to middle-aged athletes/trainees seeking to improve tendon health, reduce injury risk, or enhance athletic performance.

The experimental design of both arms will be the same, however Arm 1 will be health, middle-aged male participants and Arm 2 will be health, middle-aged female participants.

Arm/Group Information

Arm 1:

Training and nutrition intervention for middle-aged male participants (aged 35 - 59 years) who consumed hydrolyzed collagen (HC) or a placebo beverage with progressive resistance training.

Arm 1 Description:

This arm was designed as a double-blind, randomized control study with parallel groups. Middle-aged, recreationally trained men, pair-matched by age, body mass, height and baseline strength, were randomly allocated to the collagen (COL) or placebo (PLA) group.

During their initial laboratory visit, participants were screened and provided with a 3-day food diary and its completion instructions. Before undergoing baseline assessments, participants engaged in a warm-up routine that included 5 minutes of low-intensity jogging followed by 5 minutes of dynamic stretching. The baseline assessments of lower limb muscle-tendon unit properties, lower limb strength and power were conducted at the same time of day to mitigate any potential circadian effects.

The 12-week progressive lower-limb resistance training program comprised a combination of supervised and home-based training sessions focused on overloading the quadriceps femoris muscle-tendon unit. Training loads were adjusted weekly based on the prior session's performance using a linear progression model.

Each session was accompanied by the consumption of a flavor matched beverage. This beverage contained either 0 or 30 grams of hydrolyzed collagen (HC), combined with vitamin C. The 0 g beverage also contained vitamin C and was matched with the 30 g beverage for caloric content by the inclusion of maltodextrin.

Post-intervention, participants returned to the laboratory 72 hours after their final training session to repeat all baseline assessments.

Arm 2:

Training and nutrition intervention for middle-aged female participants (aged 35 - 59 years), who consumed hydrolyzed collagen (HC) or a placebo beverage with progressive resistance training.

Arm 2 Description:

This arm was identical to Arm 1 (male cohort), however it was conducted in middle-aged females.

1. **Period**

Arm 1: Participants were recruited from university staff, and sports and leisure clubs. Recruitment began in June 2021 and data collection was completed in December 2021.

Arm 2: Participants were recruited from university staff and sports clubs. Recruitment began in March 2023 and data collection was completed in June 2023.

2. **Baseline Characteristics**

Arm 1: 20 middle-aged males (mean ± SD; age, 47 ± 5 years, body mass, 81 ± 12 kg; height 178 ± 7 cm)

Arm 2: 22 middle-aged females (mean ± SD; age 37 ± 2 years, body mass, 69 ± 8 kg, 168 ± 4 cm)

- Age
    - > 35 and < 59 years;
- Sex
    - Male and female
- Ethnicity
    - Race and ethnicity information not collected
- Region of enrolment
    - Arm 1: Carlow, Ireland
    - Arm 2: Dublin, Ireland

3. Outcome measure information

Table 1. Outcome measures by study arm

	Number of participants analyzed	Description	Time frame
Primary	42 (Arm1: 20; Arm 2: 22)	m. vastus lateralis thickness (in millimeters)	Data analyzed between January 2022 & September 2023
Primary	43 (Arm1: 20; Arm 2: 22)	m. vastus lateralis fascicle pennation angle (in degrees)	Data analyzed between January 2022 & September 2023
Primary	43 (Arm1: 20; Arm 2: 22)	Patellar tendon cross sectional area (in squared millimeters) at 3 regions along the tendon length	Data analyzed between January 2022 & September 2023
Primary	43 (Arm1: 20; Arm 2: 22)	Bilateral vertical countermovement jump height (in centimeters)	Data analyzed between January 2022 & September 2023
Primary	Arm 1: 20	Patellar tendon stiffness (in Newtons per millimeter)	Data analyzed between January 2022 & September 2023

Primary	Arm 1: 20	Knee extensor maximal isometric torque (in Newton meters)	Data analyzed between January 2022 & September 2023
Primary	Arm 1: 20	Knee extensor rate of torque development (in Newton meters per second)	Data analyzed between January 2022 & September 2023
Primary	Arm 1: 20	Barbell back squat 10-repetition maximum (in kilograms)	Data analyzed between January 2022 & September 2023
Primary	Arm 1: 20	Bilateral horizontal broad jump distance (in centimeters)	Data analyzed between January 2022 & September 2023
Primary	Arm 2: 22	Lower limb maximal isometric force (in Newtons)	Data analyzed between July 2023 & September 2023
Primary	Arm 2: 22	Lower limb isometric rate of force development (in Newtons per second)	Data analyzed between July 2023 & September 2023
Primary	Arm 2: 22	20-metre sprint time (in seconds)	Data analyzed between July 2023 & September 2023

Statistical Analyses Plan (SAP)

In Arm 1, data from 20 male participants were analyzed and in Arm 2, data from 22 female participants were analyzed. The analyses of data collected from each arm were performed independently. All variables were ratio data, and normal distribution was assessed using the Shapiro-Wilk test, along with visual inspections of histograms, Q-Q plots, and box plots. All data met the assumptions of the parametric statistical analyses used in the study. Independent t-tests were used to assess differences between groups (COL, collagen and PLA, placebo) at baseline for all measures. The two-way analysis of variance (ANOVA) was performed on all measures taken at baseline and after the intervention to assess the main effects of group (COL vs PLA), time (pre- vs post- intervention), and any group × time interactions. The only exceptions to this were for patellar tendon cross-sectional area, and rate of torque/force development. For the patellar tendon cross-sectional, a three-way ANOVA was used to assess main effect of location (along the length of the tendon), and any interactions between location, time (pre- to post- intervention) and group (COL vs PLA). Likewise for rate of torque/force development, a three-way ANOVA was used to assess main effect of time-window (0 – 50, 50 – 100, 100 – 150 ms after the onset of torque/force), time (pre- to post- intervention) and group (COL vs PLA) and any interactions between time-window, time and group. When significant main effects or interaction effects were observed, paired t-tests were used for post hoc pairwise comparisons. The level of statistical significance was set at $P < 0.05$

4. Adverse Event Information

During data collection period, any adverse event such as all-cause mortality or serious adverse event did not occur.

5. Certain Agreements

Are all PIs Employees of Sponsor? Yes

Results Disclosure Restriction on PI(s)?

No

6. Results Point of Contact

Dr Rob Erskine Research Institute for Sport and Exercise Sciences, Liverpool John Moores University Tel: +44 (0)151 904 62; E-mail: R.M.Erskine@ljmu.ac.uk

Informed Consent Form

Please note that this contains legacy information, as the institution granting ethical approval (Institute of Technology, Carlow) is now known as South East Technological University. Ethical approval for this study was granted on 03/24/2021.

CONSENT TO PARTICIPATE IN RESEARCH

The Effect of Vitamin C-enriched Collagen Combined with Resistance Training on Muscle-tendon Unit Properties in Middle-aged Men and Women

You are being invited to participate in a research study about the adaptations that occur in our body after 10 weeks of resistance exercise combined with Vitamin C-enriched Collagen supplementation. We will be observing the effect of a progressive resistance intervention combined with vitamin c enriched collagen supplementation on changes in lower limb strength measured by dynamometry, as well as muscle and tendon properties measured using ultrasound. This study is being conducted by Christopher Nulty, Lecturer from the Department of Health and Sport Sciences at Institute of Technology Carlow (herein referred to as IT Carlow). Kieran Phelan, Masters by research student in the department, will assist in conducting this study as part of a MSc dissertation.

Your participation in this study is entirely voluntary. Please read the information below and ask questions about anything you do not understand, before deciding whether to participate.

You have been asked to participate in this study because this research is specifically looking at the effects of collagen supplementation with resistance training in middle-aged adults. You have met the criteria outlined in the participant information sheet and expressed interest.

- ***PURPOSE OF THE STUDY***

The purpose of this study is to investigate the changes that occur in the middle-aged muscle and tendon when supplementing with vitamin C-enriched collagen throughout a progressive resistance exercise program.

- ***PROCEDURES***

If you volunteer to participate in this study, you will be asked to be available and perform the following:

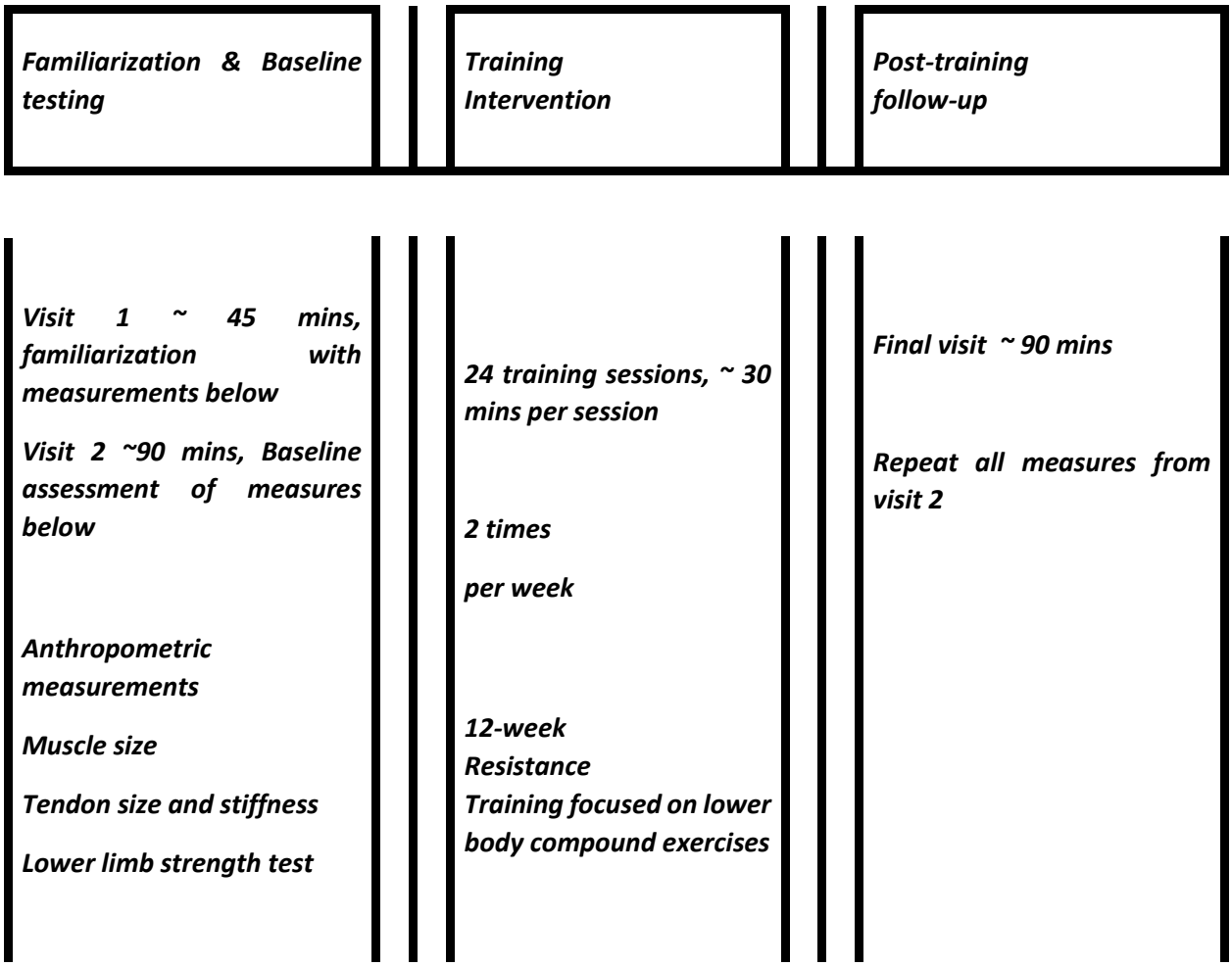

Figure 1. An overview of the study intervention periods and measurements. For further information please consult the participant information sheet

- ***POTENTIAL RISKS AND DISCOMFORTS***

You will learn the correct technique for each exercise during the familiarization session. A standard warm-up, sufficient rest periods and controlled range of motion will be provided. The exercises will be immediately ceased if participants feel uncomfortable. You are likely to experience mild discomfort

and stiffness known delayed onset muscle soreness (DOMS) in 24 – 72 hours after the first training session. This is a normal response to resistance exercise and should lessen over time.

In the event of physical and/ or mental injury resulting from participation in this research project, IT Carlow does not provide any medical, hospitalization or other insurance for participants in this research study, nor will IT Carlow provide any medical treatment or compensation for any injury sustained as a result of participation in this research study, except required by law.

- ***POTENTIAL BENEFITS TO SUBJECTS AND/ OR TO SOCIETY***

This study will provide you the opportunity to partake in a supervised exercise program designed by sport scientists. It is expected that your strength and muscle size will be increased, and your tendon health improved regardless of whether you are in the collagen group or not. As the training will be performed in groups, there may be benefits to mental health, stress and anxiety and you may make social connections with people of similar interests. The findings of this study will be made available through scientific communication, and will be of benefit to middle-aged trainees seeking to improve tendon health, reduce injury risk, and or enhance exercise performance

- ***CONFIDENTIALITY***

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law. Each participant will be allocated a personal ID. The only place the participants name and code will be together will be on the Informed Consent and an electronic spreadsheet (Excel). This spreadsheet will be stored in the IT Carlow G-drive storage facility with password-controlled access. All data will be deleted from other storage devices once transferred to a password-controlled IT Carlow computer. The researcher will deliver and collect the questionnaires and consent form on a one-to-one basis. Any data for the consent forms be only shared with my supervisors. The only identifiable information will be the consent form, which will be kept in a locked filing cabinet within the IT Carlow Research Office and an electronic (Excel) spreadsheet stored only in the IT Carlow G-drive storage facility with password-controlled access.. Confidentiality may be broken if there is a strong belief that there is a serious risk of harm or danger to either the participant or another individual (e.g. physical, emotional or sexual abuse, concerns for child protection, rape, self-harm, suicidal intent or criminal activity) or if a serious crime has been committed.

The Institute of Technology Carlow is committed to protecting the rights and privacy of individuals with respect to the processing of their personal data. A copy of the Institute's Privacy notice is available on the Institute's website (https://www.itcarlow.ie/resources/data-protection.htm). This website also contains further information relating to your rights regarding subject access requests, records retention and data protection in general. Any further queries in relation to the GDPR can be addressed to the Institute's Data Protection Oversight Group (e-mail: gdpr@itcarlow.ie)

- ***PARTICIPATION AND WITHDRAWAL***

Participation is entirely voluntary. If you volunteer to be in this study, you can withdraw at any time by informing the investigators without giving a reason and without it affecting your rights/any future treatment/service you receive.

The investigator(s) may withdraw you from this research if circumstances arise which warrant doing so. This situation may arise if there is a strong belief that there is a serious risk of harm or danger to either the participant or another individual (e.g. physical, emotional or sexual abuse, concerns for child protection, rape, self-harm, suicidal intent or criminal activity) or if a serious crime has been committed. Additionally, you may be withdrawn if you do not adhere to the study protocol. If you do decide to take part, you will be asked to sign this consent form.

- ***COMPENSATION FOR PARTICIPATION***

You will not receive any payment or other compensation for participation in this study. There is also no cost to you for participation.

- ***IDENTIFICATION OF INVESTIGATORS***

If you have any questions about the study, please do not hesitate to contact:

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- ***RIGHTS OF RESEARCH PARTICIPANTS***

IT Carlow's Research Ethics Committee has reviewed and approved our request to conduct this project (Approval no. 300). If you have any concerns about your rights in this study, please contact the Chair of the Research Ethics Committee, Dr. Brian Jackson (Brian.Jackson@setu.ie)

I understand the procedures described above. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

_____	_____
Printed Name of Participant	***Date***
_____	_____
Signature of Participant	***Date***
_____	_____
Signature of Researcher	***Date***