The Dose-response of Vitamin C-enriched Collagen on Markers of Collagen Synthesis in Middle-Aged Men and Women Following Resistance Exercise

Study protocol, statistical analysis plan and informed consent form

Document date: 17 January 2024

1. Participant Flow

The number of participants who started and completed this study is shown in Figures 1, and 2.

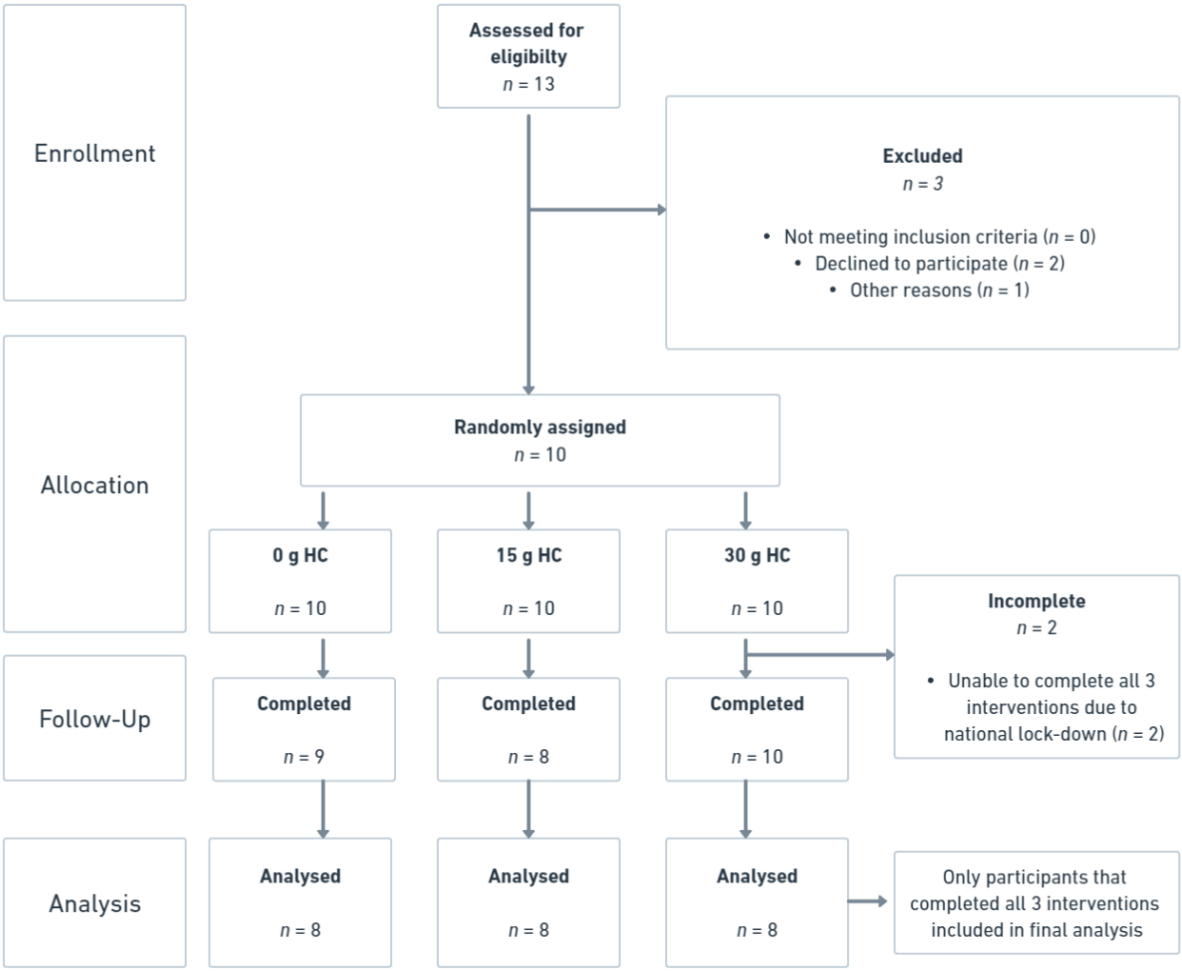

Figure 1. CONSORT flow diagram for middle-aged males. *HC*, hydrolysed collagen.

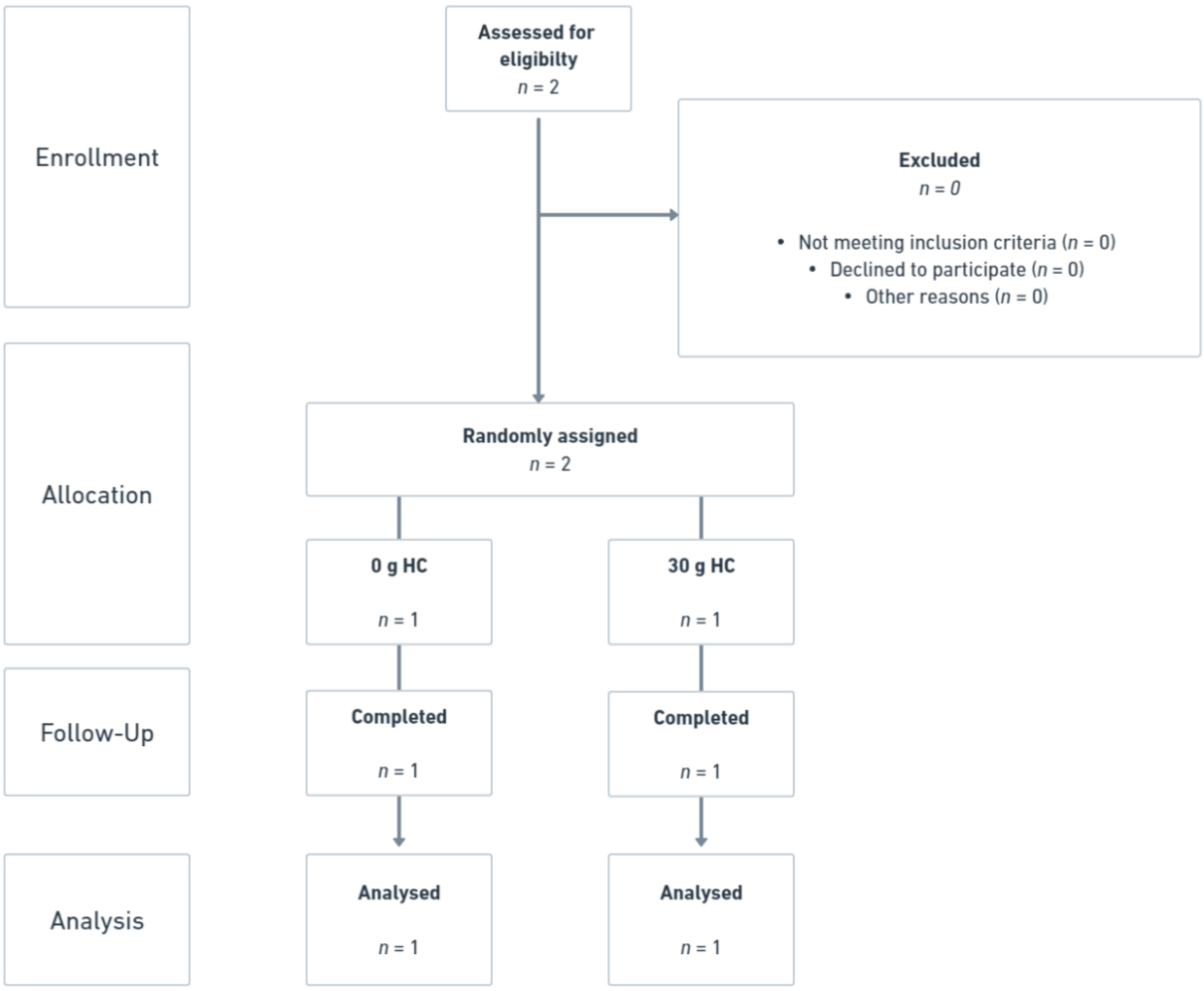

Figure 2. CONSORT flow diagram for middle-aged females. *HC*, hydrolysed collagen.

Arm/Group Information

Arm 1: Intervention for middle-aged male participants (aged 40 – 65 years): Consuming hydrolyzed collagen (HC) with resistance exercise.

Arm 1 Description:

Middle-aged male participants were asked to visit the laboratory on three different occasions to consume one of three different doses of hydrolyzed collagen (HC) and performing resistance exercise (RE). Different doses of HC (0 grams, 15 grams and 15 grams) and 50 mg vitamin C were dissolved with 250 mL water in an opaque drinks bottle. To match calories of 30 grams HC, 34.1 grams and 15.4 grams' maltodextrin were used in the 0 grams HC and 15 grams HC interventions, respectively. Also, to mask any potential difference in HC doses, 4 grams' non-caloric sweetener was included in all HC doses. Each intervention (with a seven-day washout period) lasted for seven hours and immediately after participants consumed the HC, they performed four sets of ten repetitions of leg press exercise at 10-repetition maximum load and then rested. Ten x 5 mL blood samples were collected as follows: at rest immediately prior to HC ingestion, 0.5 hours post HC ingestion, 1 hour post HC ingestion, 0.5 hours post exercise, 1 hour post exercise, 2 hours post exercise, 4 hours post exercise and 6 hours post exercise. All interventions were performed at the same time of day (08:00 - 15:00).

Blood samples were analysed to measure the concentration of a biomarker of collagen synthesis (serum procollagen type I N-terminal propeptide (PINP)), a biomarker of collagen breakdown (plasma β-isomerized C-terminal telopeptide of type I collagen (*β*-CTX)), serum

collagen amino acid concentration, and concentration of 17β-estradiol (for female participants only).

The details of nutritional supplements used are as follows:

Hydrolyzed collagen (Myprotein, Cheshire, UK), vitamin C powder (Holland and Barrett Retail Limited, Warwickshire, UK), maltodextrin (Myprotein, Cheshire, UK), and non-caloric sweetener (Truvia®, SilverSpoon, London, UK).

Arm 2: Intervention for middle-aged female participants (aged 40 – 65 years): Consuming HC with RE.

Arm 2 Description:

The intervention procedure is exactly the same as Arm 1 except for the number of visits. Middle-aged female participants visited the laboratory on three occasions across two consecutive menstrual cycles. The first visit (familiarisation) took place at the onset of menses. Following this, there were two interventions within a single month, where female participants' estrogen level was lower (i.e., at the onset of menses) or higher (i.e., at ovulation). Dates for the interventions were based on the self-reported onset of menses and previous menstrual cycle length.

Recruitment Period

Participants were recruited from the local area (Carlow, Ireland). Recruitment began in November 2019 and data collection was completed in September 2021.

2. Baseline Characteristics

Arm 1: Eight middle-aged men (mean ± SD: age, 49 ± 8 years; height, 178 ± 2 cm; body mass, 90 ± 4 kg).

Arm 2: Two middle-aged women. One participant (age, 43 years; height, 160.5 cm; body mass 63.5 kg) was a European masters level karate athlete, while the other was a recreational distance runner (age, 42 years; height, 172.6 cm; body mass 69.4 kg).

- Age
    - Middle-aged participants aged 40 to 65 years
- Sex
    - Male and female
- Ethnicity
    - Race and ethnicity information not collected
- Region of enrolment
    - Carlow, Ireland

Variables

Table 1. Primary and secondary outcome measures for each arm of the study.

Variables	Study Arm	Outcome measure type	Number of participants analysed
Serum PINP	1 and 2	Primary	10
Plasma β-CTX	1 and 2	Primary	10
Serum amino acid concentration	1 and 2	Secondary	10
Serum 17β-estradiol	2	Secondary	2

3. Outcome Measure Information

Table 1 shows data for each primary and secondary outcome measure by arm.

Outcome Measure Title and description

- Concentration of serum procollagen type I N-terminal propeptide (PINP) (µg/L)
- Concentration of plasma β-isomerized C-terminal telopeptide of type I collagen (β-CTX) (µg/L)
- Concentration of 18 amino acids that constitute collagen (µmol/L):

  Glycine, proline, hydroxyproline, glutamic acid, alanine, arginine, aspartic acid, lysine, serine, leucine, valine, phenylalanine, threonine, isoleucine, histidine, tyrosine, methionine, and glutamine.
- Concentration of 17β-estradiol (ng/L)

Outcome Measure Time Frame

Outcome measures (table 2) were analysed from August 2022 to November 2022.

Arm 1 Information

Arm 1 title: Middle-age male intervention

Arm 1 description:

Thirteen men volunteered for this study. Three withdrew prior to the commencement of the first intervention. Two participants completed a portion of the study in March 2020, however, due to immediate laboratory closure during the COVID-19 lockdown restrictions, they could not complete all interventions and were subsequently excluded from the analysis.

Arm 2 Information

Arm 2 title: Middle-aged female intervention

Arm 2 description:

Fifteen women volunteered for this study. Two female participants completed familiarisation and a single intervention each for this study before COVID-19 national lockdown in March 2020. The remaining volunteers no longer met the inclusion criteria or declined to participate once laboratories re-opened in Ireland in July 2021.

In Arm 1, data from 8 male participants were analysed and in Arm 2, data from 2 female participants were analysed.

Outcome Measure Data Table

Table 2. Outcome measure type and measure of dispersion by arm.

Variable	**Arm 1** Measure type	Measure of dispersion
Serum PINP (µg/L)	Mean	Standard deviation
Plasmaβ-CTX (µg/L)	Mean	Standard deviation
18 Serum amino acids (µmol/L)	Mean	Standard deviation
	Arm 2	
Serum PINP (ng/mL)	n/a	n/a
Plasmaβ-CTX (µg/L)	n/a	n/a
18 Serum amino acids (µmol/L)	n/a	n/a
Serum 17β-estradiol (ng/L)	n/a	n/a

Statistical Analyses

Due to the sample size (n=2) in Arm 2, only data from Arm 1 were analysed with statistical models. The data from Arm 2 were analysed descriptively.

Two-way within-subject ANOVA models (dose × time) were performed to detect changes in serum PINP, amino acid and plasma β-CTX concentration. One-way repeated measures ANOVA models were performed to detect differences in the area-under-the-curve between interventions for PINP and each of the 18 amino acids. If an interaction effect or a main effect of HC dose existed, least significant difference post-hoc pairwise comparisons were performed. The level of statistical significance was set at $P < 0.05$.

4. Adverse Event Information

During the 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: The principal investigator is an employee of the sponsor

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

Informed Consent Form (Human Tissue Act 2004)

Study Title:	*The dose-response of vitamin C-enriched collagen on markers of collagen synthesis in healthy middle aged men and women following resistance exercise*
LJMU Ethics code:	
Name of Principal Investigator:	Chris Nulty
Faculty & School:	School of Sport and Exercise Sciences
Contact details:	C.Nulty@2019.ljmu.ac.uk

Please Initial

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights. ☐
3. I understand that any personal information collected during the study will be anonymised and remain confidential. ☐
4. I consent to my blood samples being stored securely at LJMU for the duration of this ethically approved research and used for the purposes outlined in the Participant Information Sheet. ☐
5. I give the consent for my blood samples to be used for DNA analysis or other genetic testing as described in the Participant Information Sheet. ☐
6. I agree for my blood samples to be stored for the purposes of this ethically approved research and future projects under the regulation of the Human Tissue Act. ☐
7. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Researcher	Date	Signature
Name of Person taking consent *(If different from researcher*	Date	Signature

Note to researcher: *When completed one copy to be retained by the participant. A second copy to be delivered to the Human Tissue Coordinator, once the sample has been entered into the Pro-Curo database and the printed sample label affixed to the top right corner of this form and associated sample tube. Please retain a photocopy for your records in a locked filing cabinet or a scanned copy in the Hu.Tissue NAS virtual Project Folder.*

FORM: Informed Consent - Human Tissue (HT-CONSENT-003)	Approved by	
	Date effective	
	Version	
	Date of next review	