The Dose-response of Vitamin C-enriched Collagen on Markers of Collagen Synthesis in Healthy Young and Older Men and Women Following Resistance Exercise

Study protocol, statistical analysis plan and informed consent form

Document date: 06/06/2023

1. Participant Flow

The number of participants who started and completed this study is shown in Figures 1, 2, 3, and 4.

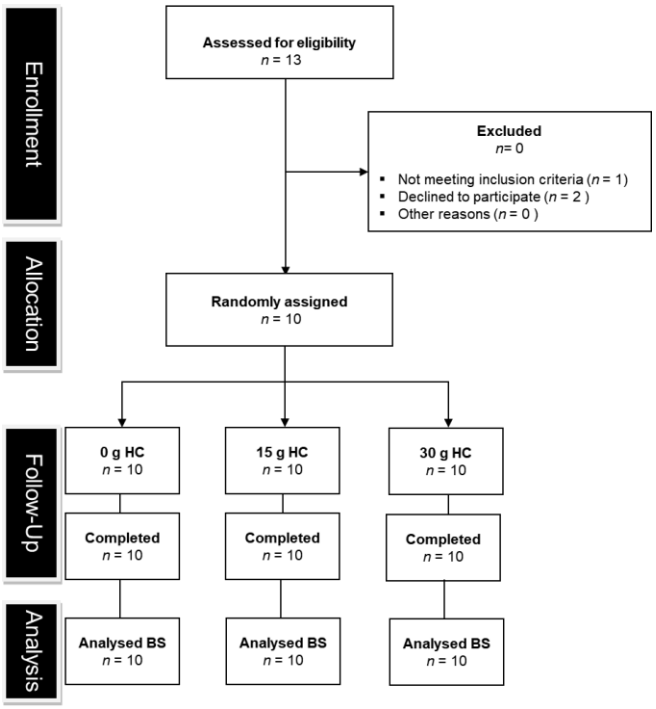

Figure 1. A CONSORT flow diagram for young males**.**

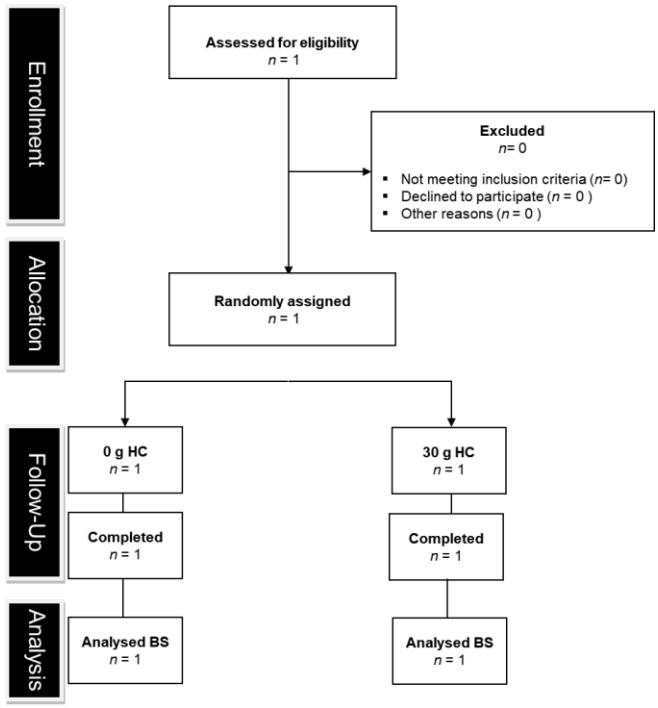

Figure 2. A CONSORT flow diagram for young females**.**

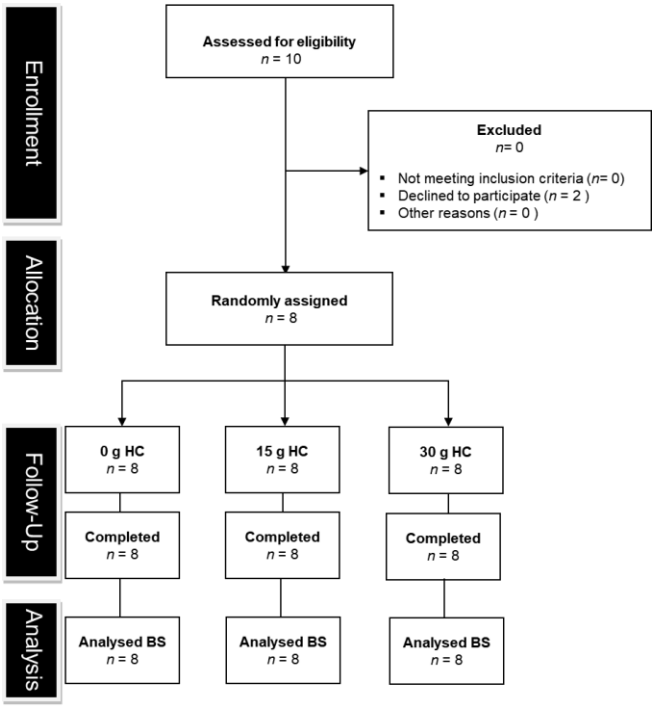

Figure 3. A CONSORT flow diagram for older males**.**

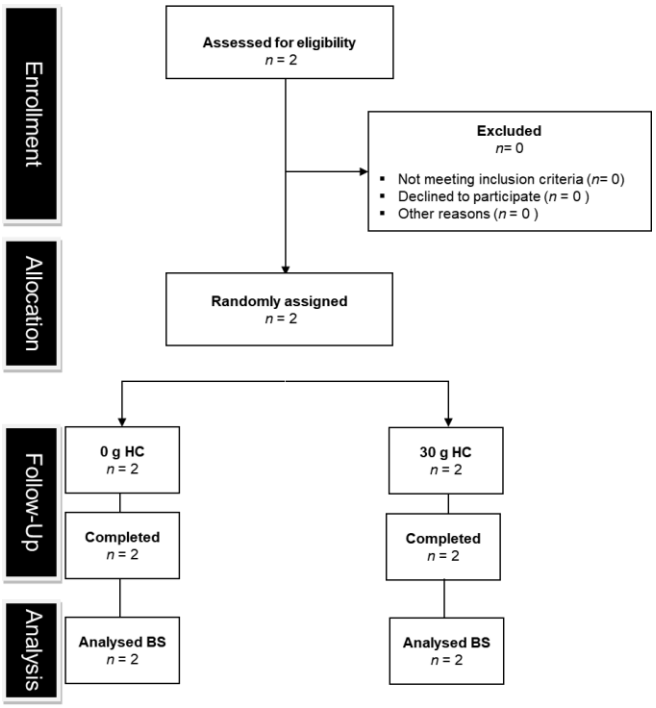

Figure 4. A CONSORT flow diagram for older females.

Arm/Group Information

Arm 1:

Trial for young male participants (aged 18 – 39 years): Consuming hydrolyzed collagen (HC) with resistance exercise

Arm 1 Description:

Young male participants were asked to visit the laboratory on three different occasions to consume one of 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 bottle. To match calories of 30 grams HC, 34.1 grams and 15.4 grams maltodextrin was used in 0 grams HC and 15 grams HC, respectively. Also, to mask any potential difference in HC doses, 4 grams non-caloric sweetener was included in all HC

doses.

Each trial (with a seven-day washout period) lasted for seven hours and immediately after participants consumed HC, they performed four sets of ten repetitions of barbell back squat exercise at 10-repetition maximum load and rested. Ten x 5 mL blood samples were collected as follows: at rest immediately prior to HC ingestion, 0.5 hour post HC ingestion, 1 hour post HC ingestion, 0.5 hour post exercise, 1 hour post exercise, 2 hours post exercise, 4 hours post exercise and 6 hours post exercise. All trials 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:

Trial for young female participants (aged 18 – 39 years): Consuming HC with RE.

Arm 2 Description:

The intervention procedure is exactly same as Arm 1 except for the number of visits. Young female participants visited the laboratory on four occasions during two consecutive months. Therefore, there were two trials in each month, where female participants' estrogen level was lower (i.e., onset of menses) or higher (i.e., ovulation). Dates for the trials were determined

based on self-report of onset of menses and previous menstrual cycle length.

Arm 3:

Trial for older male participants (aged 40 – 65 years): Consuming HC with RE.

Arm 3 Description:

The intervention procedure is the same as Arm 1.

Arm 4:

Trial for older female participants (aged 40 – 65 years): Consuming HC with RE.

Arm 4 Description:

The intervention procedure is the same as Arm 2.

Period

Period title:

Overall study

Participants were recruited from a university student population (young participants) and from the local area (older participants). Recruitment began in January 2019 and data collection was completed in September 2021.

2. Baseline Characteristics

Arm 1: 10 young men (mean ± SD; age: 26 ± 3 years, height: 1.77 ± 0.04 m, body mass: 79.7 ± 7.0 kg).

Arm 2: One young woman (age, 36 years; height, 1.61 m; body mass, 82.6 kg).

Arm 3: Eight older men (mean ± SD: age, 49 ± 8 years; height, 178 ± 2 cm; body mass, 90 ± 4 kg).

Arm 4: Two older 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
    - young participants > 18 and < 40 years;
    - older participants > 40 and < 65 years
- Sex
    - male and female
- Ethnicity
    - Race and Ethnicity Information Not Collected
- Region of Enrollment
    - Liverpool, United Kingdom

Variables	Outcome measure type	Number of participants analyzed
	Arms 1 and 3	
Serum PINP	Primary	18
Plasma β-CTX	Primary	18
Serum amino acid concentration	Primary	18

Arms 2 and 4		
Serum PINP	Primary	3
Plasma β-CTX	Primary	3
Serum amino acid concentration	Primary	3
Serum 17β-estradiol	Secondary	3

3. Outcome Measure Information

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

Table 1. Data for outcome measures.

Outcome Measure Title and description

Concentrations of serum procollagen type I N-terminal propeptide (PINP) (ng/mL)

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 (pg/mL)

Outcome Measure Time Frame

Outcome measures were analyzed from September 2019 to August 2022

Arm 1 Information

Arm 1 title:

Young male trials

Arm 1 description:

Thirteen male participants volunteered for this study but three were excluded prior to participation due to not meeting the inclusion criteria (n = 1) and declining to proceed with participation (n = 2). Therefore a total of 10 male participants completed the study, which included three separate trials.

Arm 2 Information

Arm 2 title:

Young female trials

Arm 2 description:

One female participant completed this study, which included four separate trials.

Arm 3 Information

Arm 3 title:

Older male trials

Arm 3 description:

Ten male participants volunteered for this study but two were excluded prior to participation due declining to proceed with participation. Therefore a total of eight male participants completed the study, which included three separate trials.

Arm 4 Information

Arm 4 title:

Older female trials

Arm 4 description:

Two female participants completed this study, which included four separate trials.

Analysis Population Information

In Arm 1, data from 10 male participants were analyzed and in Arm 2, data from one female participant were analyzed. In Arm 3, data from 8 male participants were analyzed and in Arm 4, data from two female participants were analyzed.

Outcome Measure Data Table

Outcome measure data are shown in Table 2.

Table 2. Outcome Measure Data.

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

(ng/mL)		
Plasma β-CTX (µg/L)	n/a	n/a
18 Serum amino acids (µmol/L)	n/a	n/a
Serum 17β-estradiol (pg/mL)	n/a	n/a
Arm 3		
Serum PINP (ng/mL)	Mean	Standard deviation
Plasma β-CTX (µg/L)	Mean	Standard deviation
18 Serum amino acids (µmol/L)	Mean	Standard deviation
Arm 4		
Serum PINP (ng/mL)	n/a	n/a
Plasma β-CTX (µg/L)	n/a	n/a
Serum 18 amino acids (µmol/L)	n/a	n/a
Serum 17β-estradiol (pg/mL)	n/a	n/a

Statistical Analyses

Due to the sample size (n=1) in Arm 2, only data from Arm 1 were analyzed.

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 area-under-the-curve between trials for PINP and each of the 18 amino acids. If an interaction effect or a main effect of HC dose existed, Bonferroni post-hoc pairwise comparisons were performed. 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?

No: The principal investigator is a PhD student, not an employee of the sponsor

Results Disclosure Restriction on PI(s)?

No

6. Results Point of Contact

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Informed Consent Form (Human Tissue Act 2004)

Study Title:	*The dose-response of vitamin C-enriched collagen on markers of collagen synthesis in healthy young and older men and women following resistance exercise*
LJMU Ethics code:	18/SPS/059
Name of Principal Investigator:	Joonsung Lee
Faculty & School:	School of Sport and Exercise Sciences
Contact details:	J.Lee1@2017.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 understand that providing a muscle biopsy is optional and thus, I do not have to provide a muscle to take part in the study. ☐
5. I consent to my skeletal muscle and blood samples being stored securely at LJMU for the duration of this ethically approved research and used for the research purposes outlined in the Participant Information Sheet. ☐
6. I give the consent for my skeletal muscle and blood samples to be used for DNA analysis or other genetic testing as described in the Participant Information Sheet. ☐
7. I agree for my skeletal muscle and blood samples to be stored for the research purposes this ethically approved research and future projects under the regulation of the Human Tissue Act. ☐
8. I agree to my GP being informed of my participation in the study (if appropriate). ☐
9. I agree to take part in the above study. ☐

Name of Participant Date Signature

Name of Researcher Date Signature

Name of Person taking consent Date Signature

(If different from researcher

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