# The effects of resistance exercise and hydrolysed collagen supplementation on changes in collagen synthesis and muscle-tendon properties in middle-aged athletes

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# Abstract

Tendon comprises approximately 70 % type I collagen and is responsible for force transmission from muscle to bone, thus its efficiency is crucial for rapid force production. Human tendons, such as the Achilles and patellar tendons, are mechanosensitive to exercise conferring a high level of mechanical strain, such as resistance exercise (RE), which stimulates type I collagen synthesis. When repeated, chronic RE, i.e. resistance training (RT), leads to an increase in both the cross-sectional area (CSA) and stiffness of the tendon, however, the magnitude of change is often lower in both female and older populations compared to young males.

Considering the high collagen content of tendon, dietary supplementation with collagen has been suggested as a strategy to augment training adaptation. It has very recently been shown that RE with supplementation of 30 g, but not 15 g, hydrolysed collagen (HC) acutely increases whole body type I collagen synthesis in young, resistance trained men to a greater extent than RE alone. Similar benefits were observed following RE with 30 g HC ingestion in a young, female athlete. However, the positive effect was diminished when circulating oestrogen was high compared to when it was low, thus suggesting collagen synthesis varies according to menstrual cycle phase. Furthermore, recent studies have shown that RT and HC supplementation promote greater tendon adaptations (e.g. augmented CSA and stiffness) compared to RT alone. However, all these studies have been performed in young adults, and it remains to be seen if similar benefits can be seen in older individuals, who tend to demonstrate diminished anabolic responses to protein ingestion. Furthermore, studies investigating the effect of ageing on muscle-tendon health and function focus on the extremes of the adult age spectrum, i.e. neglecting middle-aged individuals. This

means that exercise and nutrition guidance for optimal tendon health in this population is lacking. Therefore, the overall aim of this thesis was to investigate the effects of RE with HC ingestion on markers of collagen turnover, and to examine the effect of HC supplementation on muscle-tendon adaptations to chronic RT in middle-aged men and women.

In order to ascertain whether HC supplementation is required in middle-aged individuals, and if a potential requirement is sex-dependent, we first needed to determine the collagen composition of habitual diets in adults of different ages. Aside from one recent study on the American adult population (with substantial methodological flaws), collagen intake at population level is yet to be described. **Chapter Three** comprehensively estimated habitual collagen intake among Irish adults using data from the National Adult Nutrition Survey (NANS), the most recent population-level data for Ireland, analysing 4-day semi-weighed food diaries from 1500 adults. The collagen mean daily intake for the entire sample was 3.2 ± 2.0 $g \cdot day^{-1}$, representing just 3.6 ± 1.9 % of total protein intake. These findings suggest that, if collagen intake is beneficial for musculoskeletal health, sufficient quantities are difficult to obtain through habitual diet alone, and thus supplementation is likely warranted, especially in ageing and female populations, who had the lowest intakes.

The first lab-based experimental study of this thesis (**Chapter Four**) used a double-blind, randomised crossover design to determine the optimal dose of HC for maximising whole-body collagen synthesis in eight middle-aged, resistance trained men. Venous blood samples were analysed for respective markers of collagen synthesis and degradation, the N-terminal propeptide of type 1 pro-collagen (PINP), and β-isomerized C-terminal telopeptide of type 1 collagen (β-CTx), as well as postprandial serum aminoacidemia regarding 18 collagen amino acids. Strikingly,

serum PINP did not increase following RE alone. However, both 15 and 30 g HC ingested prior to RE elevated the serum PINP concentration × time area-under-the-curve (AUC). The PINP AUC was even higher when 30 g HC was ingested compared to 15 g HC (169 ± 28 vs. 134 ± 23 μg/mL × h, $P < 0.05$), and this effect was mirrored in higher amino acid serum concentration × time AUCs regarding glycine, proline, and hydroxyproline (amongst other collagen amino acids). Although β-CTx decreased immediately following RE, there was no additional effect of HC ingestion on this marker of collagen degradation.

The second lab-based experimental study (**Chapter Five**) extended this work to middle-aged, eumenorrheic, premenopausal women. In this case study, two resistance trained women performed 4 sets of high intensity RE in the late follicular phase of their menstrual cycles, where one participant ingested 30 g HC, and the other an energy matched placebo (0 g HC). The findings were consisted with the male cohort, i.e. there was no change in serum PINP concentration in the 6 h following RE with 0 g HC. However, the participant who ingested 30 g HC experienced a noticeable peak in PINP 4 h post-RE (37.4 μg/mL, approximately 2-fold baseline), and a greater concentration × time AUC (193 μg/mL × h) compared to the participant who ingested the 0 g beverage (85 μg/mL × h). These findings were also consistent with the bioavailability of 18 collagen amino acids, i.e. the serum concentration × time AUCs were low during the 0 g HC intervention but high during the 30 g HC intervention.

Based on the findings of chapters four and five, showing that 15 g recovers the collagen synthesis response to RT in middle-aged men, but that 30 g HC is most effective at increasing post-RE collagen synthesis in middle-age, resistance trained men and women, the third (**Chapter Six**) and fourth (**Chapter Seven**) experimental

chapters explored the chronic effects (8 – 12 weeks) of RT with 30 g HC on patellar tendon adaptations in middle-aged women and men, respectively.

**Chapter Six** investigated the effects of eight weeks' eccentric RT with 30 g HC supplementation on changes in tendon properties in premenopausal, middle-aged female athletes. Specifically, 22 international Master field hockey athletes were recruited from the Ireland over 35s and over 40s squads, and randomly assigned to either a collagen (COL, n = 10) or placebo (PLA, n = 12) group in a double-blind manner. Participants ingested either 30 g HC (COL) or 30 g maltodextrin (PLA), both with 500 mg vitamin C, three times a week for eight weeks. Alongside supplementation, they completed one high-intensity flywheel squat RE session and two lower-limb eccentric bodyweight RE sessions per week, which was in addition to their regular hockey training. Both groups experienced increases in muscle size and maximal voluntary force. Patellar tendon CSA increased in both groups ($F_{1,\ 20}$ = 36.482, $p < .001$, $\eta_p^2 = 0.646$) but the 4.6 ± 2.7 % increase in COL was greater than the 2.0 ± 2.8 % increase in PLA ($t_{20} = 2.18$, $p = 0.021$, $d = 0.935$). Similarly, peak rate of force development (pRFD) increased in both groups ($F_{1,17} = 14.26$, $P = 0.002$, $\eta_p^2 = 0.456$) but the 27.3 ± 19.2 % increase in COL was greater than the 8.0 ± 19.6 % increase in PLA ($t_{1,18} = 2.16$, $p = 0.045$, $d = 0.994$).

**Chapter Seven**, the final experimental study of this thesis, involved a double-blind, parallel designed study, where 20 recreationally active men (age, 47 ± 5 years) were randomly assigned to either a placebo (PLA, n = 11) or collagen (COL, n = 9) group. Both groups performed progressive lower limb RT twice weekly for 12 weeks. Participants consumed either 30 g HC and 50 mg vitamin C (COL) or an energy-matched beverage, containing 30.5 g maltodextrin and 50 mg vitamin C (PLA). Following RT, patellar tendon CSA increased in COL but not in PLA. Patellar tendon

stiffness and Young's modulus increased more in COL (+661 ± 331 N/mm; + 0.21 ± 0.13 GPa) than in PLA (+ 247 ± 305 N/mm, group × time P = 0.009; + 0.09 ± 0.13 GPa, group × time, p = 0.018). Both groups experienced similar increases in muscle size, strength and peak rate of torque development. Although there were increases in tendon stiffness with RT alone, the lack of change in tendon CSA in PLA aligns with the potential tendon anabolic resistance suggested in **Chapter Three**, and further supports the changes in collagen turnover were mostly reflective of tendon, rather than muscle or bone.

This thesis provides the first evidence that adult populations in Ireland consume low amounts of collagen, especially females and older adults, which is likely to have implications for musculoskeletal health. Novel findings show the collagen synthesis response to a single bout of high intensity RE is blunted in middle-aged, resistance-trained men and women, but that it can be rescued by ingesting 15 g HC, while 30 g HC provides an even greater benefit.

The effect of chronic RT with 30 g HC supplementation produced positive, but slightly differing outcomes in male and female muscle-tendon adaptations, likely due to methodological differences between the studies. After 12 weeks, high intensity RT increased patellar tendon stiffness and Young's modulus, but not tendon CSA, in recreationally active, healthy, middle-aged men. Supplementing RT with 30 g HC, on the other hand, increased patellar tendon CSA and enhanced gains in tendon stiffness and Young's modulus, without translating to greater increases in pRFD. In international female Master athletes, eight weeks' eccentric RT increased patellar tendon CSA and pRFD, but these gains were augmented by ingesting 30 g HC alongside training. Taken together, the novel findings from these exercise-nutrition studies have the potential to impact training and nutrition prescription in middle-aged

athletic populations. Future studies should focus on the optimal HC dosing strategies for middle-age and older females across diverse hormonal profiles, including post-menopausal women and those prescribed exogenous hormone replacement. Given the positive effects observed on tendon properties and muscle-tendon unit function, long-term prospective studies could examine whether these adaptations lead to a reduction in injury risk in recreationally active middle-aged cohorts and Master athletes.

## Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Finally, thank you to my parents, David and Colette, for your unconditional love and support throughout the process.

## List of abbreviations

AGE: Advanced glycation end product

AKT: Protein kinase B

ANCOVA: Analysis of covariance

ANOVA: Analysis of variance

AUC: Area under the curve

BF: Biceps femoris

BIA: Bioelectrical impedance

BJ: Broad jump

BM: Body mass

BMD: Bone mineral density

BMI: Body mass index

BS: Blood Sample

CDC: Centre for Disease Control

CHO: Carbohydrate

CMJ: Countermovement jump

COFID: Composition of foods integrated dataset

COL: Collagen

CP: Collagen peptides

CSA: Cross-sectional area

β-CTX: The beta cross-linked C-terminal telopeptide of type I collagen

CV: Coefficient of variation

DXA: Dual x-ray absorptiometry

ECLIA: Electrochemiluminescence immunoassay

ECM: Extracellular matrix

EDTA: Ethylenediaminetetraacetic acid

ELISA: Enzyme-linked immunosorbent assay

EMG: Electromyography

ERT: Oestrogen replacement therapy

FM: Fat mass

FSAI: Food Safety Authority of Ireland

FSH: Follicle stimulating hormone

FSR: Fractional synthetic rate

GH: Growth hormone

HC: Hydrolysed collagen

IGF-I: Insulin-like growth factor I

IMTP: Isometric mid-thigh pull

IUNA: Irish Universities Nutrition Alliance

IV: Intravenous

KE: Knee extension

KF: Knee flexion

LBM: Lean body mass

LOX: Lysyl oxidase

LP: Luteal phase

MC: Menstrual cycle

MDI: Mean daily intake

MMP: Matrix metalloproteinases

MPS: Myofibrillar protein synthesis

MRI: Magnetic resonance imaging

MSS: Maximum sprint speed

MT: Muscle thickness

MTU: Muscle-tendon unit

MVC: Maximum voluntary contraction

MVF: Maximum voluntary force

MVIC: Maximum voluntary isometric contraction

MVT: Maximum voluntary torque

NANS: National adult nutrition survey

OC: Oral contraception

OCP: Oral contraceptive pill

OM: Onset of menses

PI3K: Phosphoinositide 3-kinase

PICP: Carboxyterminal propeptide of procollagen type I

PINP: Aminoterminal propeptide of procollagen type I

PLA: Placebo

RDA: Recommended daily allowance

RE: Resistance exercise

RFD: Rate of force development

1-RM: One repetition maximum

RMS: Root mean squared

RMVC: Ramped maximum voluntary contraction

RNA: Ribonucleic acid

RPM: Revolutions per minute

RSI: Reactive strength index

RT: Resistance training

RTD: Rate of torque development

SCP: Specific collagen peptides

SD: Standard deviation

SEM: Standard error of measurement

TGF-β: Transforming growth factor-β

mTORCI: Mammalian target of rapamycin complex I

UK: United Kingdom of Great Britain and Northern Ireland

USA: United States of America

USD: United States dollar

VL: Vastus lateralis

VLT: Velocity at lactate threshold

VM: Vastus medialis

VO2: Volume of oxygen

WP: Whey protein

YM: Young's modulus

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# Chapter One

# General Introduction

## 1.1 Introduction

Tendons are fibrous connective tissues, primarily comprised of a collagen-rich extracellular matrix, that function to transmit the force produced by skeletal muscle to bones to cause movement (Screen *et al.*, 2015). Specific tendons, such as the Achilles and patellar tendons, are crucial for the storage and release of energy during locomotion and common athletic movements like running and jumping (Komi, 1990; Magnusson *et al.*, 2003b). As such tendon properties, including stiffness, are closely related to the rate of force development, with stiffer tendons allowing for more efficient force transfer during explosive movements (Bojsen-Møller *et al.*, 2005). While the parallel collagen fibres need to resist relatively low tensile forces to avoid energy loss during most everyday tasks, movements involving rapid deceleration and high eccentric loads can exceed the maximum tensile strength of lower limb tendons (Komi *et al.*, 1992). This vulnerability is particularly evident in middle-aged individuals, in whom the incidence of Achilles, patellar, and quadriceps tendon rupture are reported to peak (Clayton & Court-Brown, 2008b). It has been suggested that soft tissue injury rates are higher in middle-aged individuals compared to older adults due to a combination of age-related tendon degeneration and higher activity levels in middle-age (Kannus & Jozsa, 1991; Clayton & Court-Brown, 2008b).

Tendons are known to be ‘mechanosensitive’, and as such the morphological and material properties of human tendon are influenced by habitual loading (Couppé *et al.*, 2008; Westh *et al.*, 2008; Heinemeier & Kjaer, 2011; Couppé *et al.*, 2021) and remodel following repeated, exercise-induced mechanical loading (Bohm *et al.*, 2015; Lazarczuk *et al.*, 2022). An acute bout of knee extensor resistance exercise (RE) increases patellar tendon collagen fractional synthetic rate (FSR) six hours after

exercise, which remains elevated above baseline (by 1-2 % per 24 hour) in the days after exercise (Miller *et al.*, 2005a). Chronically repeated RE, i.e. resistance training (RT), confers repeat exposure to high levels of mechanical strain (deformation of tendon under load), leading to increased collagen fibril density and collagen cross-linking (Couppe *et al.*, 2009). Accordingly, ~ 8-15 weeks' RT results in hypertrophy and increased stiffness of human Achilles and patellar tendons (Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009; Kubo *et al.*, 2012; Centner *et al.*, 2019; Quinlan *et al.*, 2021; Centner *et al.*, 2022a).

Age and sex influence both tendon properties and RT-induced tendon adaptations. Female tendon exhibits a lower collagen FSR than male tendon following acute RE (Miller *et al.*, 2007), and while female tendon increases in size and stiffness in response to RT, the magnitude of these adaptations are reportedly lower than in age-matched males (Magnusson *et al.*, 2007a; Burgess *et al.*, 2010; Onambele-Pearson & Pearson, 2012; McMahon *et al.*, 2018). Although the underlying mechanisms remain unclear, these sex differences may partly relate to higher circulating oestrogen levels in women, which could influence tendon adaptation through the presence of nuclear oestrogen receptors (ERα and ERβ) (Heldring *et al.*, 2007), and the inhibitory effect of oestrogen on enzymatic collagen cross-linking observed *in vitro* (Lee *et al.*, 2015). However, other biological sex differences beyond oestrogen may also contribute, including differences in body size and the lower absolute exercise loads often observed in female participants. Additionally, the use of synthetic hormones, such as those found in oral contraceptive pills (OCP), has been associated with lower tendon collagen FSR and lower collagen synthesis response to acute exercise in premenopausal women (Hansen *et al.*, 2008; Hansen *et al.*, 2009b). In contrast, oral oestrogen replacement therapy has been shown to enhance tendon collagen FSR in

postmenopausal woman (Hansen *et al.*, 2009a), and it remains difficult to isolate the effects of synthetic hormones from other hormonal and physiological differences between users and non-users**.** Nonetheless, these factors may contribute to the increased soft tissue injury risk observed in young women (Hewett *et al.*, 2016), as well as the rise in tendon rupture incidence after menopause (Hansen & Kjaer, 2016) and highlight the need for sex-specific research, as findings from male-only studies may not be directly applicable to female populations.

Additionally, ageing is associated with a reduction of collagen content in human tendon (Couppe *et al.*, 2009), greater tendon compliance (Kubo *et al.*, 2007; Eriksen *et al.*, 2018; Lindemann *et al.*, 2020; Kubo *et al.*, 2022; Létocart *et al.*, 2024) and a lower rate of force development (Quinlan *et al.*, 2018). These changes are thought to result from a combination of decreased collagen synthesis, altered cross-linking of collagen fibrils, and increased accumulation of advanced glycation end-products (AGE), which reduce the tendon's mechanical integrity and adaptability (Couppe *et al.*, 2009; Svensson *et al.*, 2017). These structural and functional impairments contribute to a decline in physical performance, reduced ability to complete tasks of daily living, and an increased risk of tendon injury in ageing populations (de Jonge *et al.*, 2011; Albers *et al.*, 2016). Despite this, older tendon appears to retain the ability to respond to RT (Reeves *et al.*, 2003b), albeit with a lower rate of adaptation compared to younger tendon in some (Quinlan *et al.*, 2021) but not all studies (Létocart *et al.*, 2024). Furthermore, due to the common use of polarised age groups (i.e. young vs. old) to demonstrate age effects, tendon adaptation to RT in middle-age remains understudied.

The combined effects of collagen supplementation with exercise have garnered recent interest, with studies providing various doses of gelatine or hydrolysed collagen (HC)

during short- and long-term exercise interventions. Previous studies have suggested positive effects of RT supplemented with HC on rate of force development (Lis *et al.*, 2021; Bischof *et al.*, 2023) and skeletal muscle size (Balshaw *et al.*, 2022). Recent evidence in young adults indicates that skeletal muscle collagen fractional synthetic rate (FSR) is unaffected by 30 g HC or whey protein supplementation (Aussieker *et al.*, 2023). Despite this, a later study by the same authors found a blend (25 g whey, 5g HC) increased myofibrillar and muscle connective tissue protein FSR at rest, but not after resistance exercise (Aussieker *et al.*, 2024b). Furthermore, one study demonstrated a dose-response relationship between HC ingestion and whole body collagen synthesis following high intensity RE in young men, where 30 g HC, but not 15 g augmented a systemic biomarker of RE-induced collagen synthesis (Lee *et al.*, 2023c), indicating that passive collagen rich tissues like tendons are the more likely target by these interventions.

With regards to longer-term RT effects, studies in middle-aged (Zdzieblik *et al.*, 2021) and older men (Zdzieblik *et al.*, 2015) reported improved strength and body composition when RT was combined with HC ingestion, although none of these studies measured tendon properties, or included female participants. It has very recently been suggested that RT with HC supplementation enhances tendon hypertrophy in young males (Jerger *et al.*, 2022; Jerger *et al.*, 2023) and tendon stiffness in young female athletes (Lee *et al.*, 2023a), although this is yet to be investigated in middle-aged populations.

When this PhD project commenced in June 2019, there were no studies investigating the acute effects of RE with collagen supplementation on markers of collagen turnover, or the effects of chronic RT with collagen supplementation on exercise-induced tendon adaptation. Since then, several studies have examined these effects, albeit exclusively

in young adults, thus the acute and chronic effects of RT with HC supplementation in middle-aged men and women are unknown. It is notable that these studies provided collagen supplements in a wide range of doses, from 5 g daily to 30 g as a single bolus taken with each RE bout. While collagen is a protein naturally present in animal products, its contribution from habitual diets is yet to be characterised. Consequently, the appropriateness of the supplementation doses used in these studies is unclear, and it is yet to be determined whether certain demographics require higher doses or can meet their needs without supplementation.

**Aims and objectives**

The overarching aims of this PhD thesis are (i) to investigate habitual collagen intake at population level; (ii) to examine the acute effects of high intensity RE with HC supplementation on markers of collagen turnover in middle-aged men and women; and (iii) to explore the effects of chronic (8 – 12 weeks), high intensity RT with HC supplementation on changes in muscle-tendon properties in middle-aged men and women. This thesis will seek to achieve these aims through completion of the following objectives:

1. To comprehensively quantify the habitual dietary collagen intake of Irish adults and identify population-specific patterns of collagen intake i.e., differences in intake by age and sex. This will be addressed in the work described in Chapter Three.

2. To determine the dose-response relationship between HC ingestion and biomarkers of collagen turnover (i.e. which HC dose provides the highest collagen synthesis response and least collagen breakdown) following an acute

bout of RE in resistance trained, middle-aged men. This will be addressed in the work described in Chapter Four.

3. To identify the effects of RE with and without 30 g HC on markers of collagen turnover during the late follicular phase of the menstrual cycle in resistance trained, middle-aged, premenopausal women. This will be addressed in the work described in Chapter Five.

4. To determine the effect of 8 weeks' high intensity eccentric RT and 30 g HC supplementation on changes in muscle-tendon properties in middle-aged female athletes. This will be addressed in the work described in Chapter Six.

5. To establish the effect of 12 weeks' high intensity RT with 30 g HC supplementation on changes in muscle-tendon properties in recreationally active, middle-aged men. This will be addressed in the work described in Chapter Seven.

# Chapter Two

# The effect of exercise with collagen supplementation on markers of collagen turnover and musculoskeletal adaptation: A narrative review of the literature

# 2.1 Introduction

Collagen constitutes approximately 30 % of total protein in humans and other mammals (Shoulders & Raines, 2009). Various tissues, including skin, muscle, bone, tendon, ligament, and blood vessels rely on their collagen-rich extracellular matrix (ECM) to provide structure (Kjaer, 2004; Shoulders & Raines, 2009; Jana *et al.*, 2019). Collagen content plays a crucial role in the efficiency of force transfer in the muscle-tendon unit (MTU). The collagen content of tendon decreases with ageing (Couppe *et al.*, 2009), which may contribute to reduced exercise performance and increased injury risk in older populations. Similarly, different sex hormone profiles between men and women seem to be associated with lower collagen synthesis in women versus men (Miller *et al.*, 2007), thereby potentially contributing to sex differences in MTU properties (Burgess *et al.*, 2010), and thus sex-differences regarding physical performance and injury risk across different age groups (Hewett *et al.*, 2016).

Although skeletal muscle is the primary force production apparatus, the collagen fibres, which are aligned in parallel bundles within the ECM of tendon, permit efficient force transfer from muscle to bone, enabling movement (Monti *et al.*, 1999). Tendons respond to mechanical strain, where an acute bout of resistance exercise (RE) increases the collagen fractional synthetic rate of tendon (Miller *et al.*, 2005a), and long-term resistance training (RT) leads to regional hypertrophy and increased stiffness and elastic modulus (Seynnes *et al.*, 2009; Quinlan *et al.*, 2021). A larger, stiffer tendon should result in greater rate of force development (RFD) (Bojsen-Møller *et al.*, 2005; Maffiuletti *et al.*, 2016; Quinlan *et al.*, 2017), enhancing explosive exercise performance in athletes, and improving quality of life through better performance in

daily living tasks of older adults e.g. stair negotiation and fall prevention (Reeves *et al.*, 2003b).

Nutritional strategies such as collagen supplementation have gained traction due to their potential role in supporting tendon health and adaptation to exercise. Seminal work in young men from Shaw *et al.* (2017) demonstrated a dose-response of collagen (gelatine) supplementation on collagen synthesis (15 g > 5 g and 0 g). Since then, studies have investigated the effects of 5 – 35 g collagen in the form of gelatine, bone broth, and hydrolysed collagen peptides (HC), with acute RE and reported aminoacidemia, markers collagen synthesis in blood, urine and skeletal muscle with mixed results (König *et al.*, 2018a; Alcock *et al.*, 2019b; Alcock *et al.*, 2019c; Clifford *et al.*, 2019; Lis & Baar, 2019; Skov *et al.*, 2019; Aussieker *et al.*, 2023; Lee *et al.*, 2023c; Robberechts *et al.*, 2023; Lee *et al.*, 2024c). Specifically, 30 but not 15 g HC, augmented collagen synthesis following a single bout of RE in young men (Lee *et al.*, 2023c) which suggests higher doses of collagen may be required to maximise connective tissue turnover after exercise. Moreover, the interaction between collagen supplementation and exercise in ageing populations is yet to be investigated.

Additionally, a small number of training studies lasting 6 – 15 weeks have now examined the effects of 5 – 30 g collagen with RT on muscle-tendon unit properties. Some have observed benefits on strength and fat-free mass (FFM) in both young and older individuals (Zdzieblik *et al.*, 2015; Jendricke *et al.*, 2019), however these findings are inconsistent (Zdzieblik *et al.*, 2015; Kirmse *et al.*, 2019a; Jacinto *et al.*, 2022b). Moreover, although HC did not influence tendon properties with RT in one study (Balshaw *et al.*, 2022), others have shown this combination to augment RT mediated tendon hypertrophy in young men (Jerger *et al.*, 2022; Jerger *et al.*, 2023) and tendon stiffness in young women (Lee *et al.*, 2023a; Lee *et al.*, 2024a). Despite

this, the effects of collagen supplementation with RT on tendon properties of ageing men and women are yet to be explored.

The aims of this narrative review are threefold: 1. to investigate the available literature on dietary collagen intake at the population level, 2. to critically appraise the literature regarding the acute collagen turnover response to exercise, with particular attention to age, sex, and supplementation effects; and 3. to critically appraise the literature regarding the chronic adaptations of tendon to RT with collagen supplementation.

# 2.2 Collagen turnover

### *Collagen structure*

Collagen is the main structural component of extracellular matrix (ECM) which makes up the main body of tendon (Screen *et al.*, 2015). All collagen molecules are characterised by repetitions of the proline-rich tripeptide (3 α chains) sequence glycine-X-Y forming a triple-helical structure (Gelse *et al.*, 2003). As glycine always occupies the first position, the X and Y positions can be occupied by any other amino acid (AA), however, they are most likely to be occupied by proline and hydroxyproline (Ramshaw *et al.*, 1998). For example, type I collagen (the fibril forming collagen most abundant in dermis, tendon, ligament and bone) has the molecular structure: $[\alpha1(I)]_2\alpha2(I)$ i.e. two identical α1(I) chains and one α2(I).

### *Collagen turnover in the extracellular matrix of musculoskeletal tissue*

*Collagen synthesis*

Fibroblasts are the primary collagen producing cells in tendon and are embedded longitudinally within the dense ECM (Gelse *et al.*, 2003; Kjær *et al.*, 2009). Collagen

synthesis is initiated intracellularly with the transcription of collagen genes into mRNA, which is then translated into pre-procollagen polypeptide chains on ribosomes along the rough endoplasmic reticulum of fibroblasts. Procollagen consists of three α chains that undergo extensive post-translational modifications, including hydroxylation of proline and lysine residues and glycosylation of hydroxylysine residues (Myllyharju, 2003). Vitamin C is utilised as an essential co-factor in these processes, which are critical for proper folding of the procollagen into a triple-helix structure (Myllyharju, 2003; Canty & Kadler, 2005; Kjaer *et al.*, 2006).

During the biosynthesis of type I collagen, procollagen is secreted into the extracellular space of tendons. Procollagen peptidases then cleave the globular N- and C-terminal propeptides (termed procollagen N-terminal propetide [PINP], and procollagen C-terminal propeptide [PICP], respectively), thereby converting procollagen into tropocollagen monomers (Leung *et al.*, 1979). Tropocollagen monomers then assemble into fibrils, and adjacent molecules form covalent bonds (known as cross-links) between lysine and hydroxylysine residues. This process is known as enzymatic cross-linking, which is catalysed by lysyl oxidase (LOX) (Gelse *et al.*, 2003) and serves to increase tensile strength and stiffness of connective tissue.

*Collagen degradation*

A family of collagenases known as matrix metalloproteinases (MMPs) are known to target the triple-helix structure of collagen, thereby mediating collagen degradation. These enzymes cleave specific α chain bonds in interstitial collagens I, II, and III generating N-terminal and C-terminal fragments that are three-fourths and one-fourth the length of the original α-chains, respectively (Fields, 2013). Among the fragments released into circulation is the beta cross-linked C-terminal telopeptide of type I

collagen (β-CTX), which is among the fragments cleaved off by cathepsin K, a lysosomal cysteine protease which attacks the telopeptides found in tendon (Zou *et al.*, 2022).

***Effect of exercise on collagen turnover***

Chronic resistance exercise (RE) induces remodelling of musculoskeletal tissues through 'mechanotransduction', leading to myofibrillar and collagen proteins in muscle, and collagen protein accretion in tendon, which in turn increases cell diameter and ultimately, increases the cross-sectional area of muscle and tendon. Despite advances in our understanding, measuring muscle and tendon collagen turnover *in vivo* remains challenging due to the invasive nature of the techniques required. The musculoskeletal tissue biopsy is widely considered the gold standard for determining the fractional synthesis rate (FSR) of connective tissue and muscle protein fractions. This measurement typically involves the infusion of stable isotope-labelled amino acids, such as l-[ring-13C6]-phenylalanine, or L-[1-$^{13}$C] proline with tissue biopsies taken in the hours to days after exercise. Although two studies using the 14C bomb pulse method in a small number of cadaverous tendons suggested minimal collagen turnover in adulthood (Heinemeier *et al.*, 2013; Zhang *et al.*, 2020), there are several reasons to challenge this view. Smeets *et al.* (2019), utilising tissue collected during total knee arthroplasty in older adults, demonstrated that tendon turnover (FSR 0.06 % per hour) is comparable to skeletal muscle turnover (0.04 % per hour). Additionally, several studies have now shown that, following a bout of RE, the FSR of both myofibrillar and muscle connective tissue fractions in muscle (Moore et al., 2005; Trommelen et al., 2020; Holm et al., 2010; Aussieker et al., 2023; Kirmse et al., 2019) and the FSR of tendon (Miller *et al.*, 2005a; Miller *et al.*, 2007) are increased. Furthermore, studies have measured collagen synthesis in serum/plasma and using the

microdialysis technique (insertion of catheter into the peritendinous space) to quantify the concentrations of aminoterminal and carboxyterminal propeptides of procollagen type I (PINP and PICP) (Langberg *et al.*, 1999b; Langberg *et al.*, 2001b; Heinemeier *et al.*, 2003; Miller *et al.*, 2005a; Miller *et al.*, 2007; Hansen *et al.*, 2009a; Hansen *et al.*, 2009b). Although often suggested that these biomarkers reflect bone collagen synthesis (Dolan *et al.*, 2022), these are, in fact, global markers of type I collagen synthesis and likely to reflect the tissue being stimulated. Clearly, collagen turnover in musculoskeletal tissues is complex, and these findings highlight the need for further research to enhance our in vivo understanding of collagen synthesis and tissue remodelling following resistance training, particularly in females with diverse hormonal profiles.

***Effect of advancing age on collagen turnover***

Ageing impacts the turnover of collagen in collagen-rich tissues, resulting in an altered turnover ratio, however the effects in human tendon are not fully understood. A consequence of ageing is the upregulation of MMPs leading to an increase in collagen breakdown in collagen rich tissues (Kragstrup *et al.*, 2011). Ageing also negatively impacts collagen synthesis, as fibroblast activity decreases with age (Gelse *et al.*, 2003; Kjær *et al.*, 2009). Recently, Gumpenberger *et al.* (2020) found no change in the expression of *COL1A1* or *MMPs* (both genes are associated with collagen turnover) in intramuscular connective tissue following RE in older men, suggesting anabolic resistance. At present, there is a lack of human data to determine whether these responses are tissue specific. Two studies have demonstrated the activity of ageing tenocytes, showing increased tendon collagen synthesis following local growth factor injections in middle-aged and older men. In these studies, middle-aged men exhibit increases in tendon FSR following injection of growth hormone (GH) (Doessing *et*

*al.*, 2010) and both young and old male patellar tendon exhibit similar tendon collagen FSR following local infusion of insulin-like growth factor-I (IGF-I, a known anabolic growth factor in tendon (Nielsen *et al.*, 2014). Nielsen *et al.* (2014) also found lower circulating IGF-I in older adults, which may at least partially explain why the collagen content of older male tendon is approximately 1/3 lower than in younger male tendon (Couppe *et al.*, 2009). It remains unknown whether tenocytes in middle-aged and older adults respond to exercise stimuli.

Advancing age is also related to changes in tendon fibril cross-linking, characterised by the accumulated increase in advanced glycation end products (AGEs) i.e., non-enzymatic cross-links (Bank *et al.*, 1999; Couppe *et al.*, 2009). AGE cross-links, such as pentosidine, are created when lysine amino acids in the collagen triple helix interact with glucose (Bailey *et al.*, 1995). Although animal models suggest the age-related accumulation of AGEs is related to increased strength, stiffness and failure load (Galeski *et al.*, 1977; Andreassen *et al.*, 1988; Reddy *et al.*, 2002; Reddy, 2004), studies in humans consistently report that tendons in older individuals are more compliant compared to those in younger individuals (Karamanidis & Arampatzis, 2006; Mian *et al.*, 2007; Carroll *et al.*, 2008; Stenroth *et al.*, 2012; Quinlan *et al.*, 2017; Quinlan *et al.*, 2021; Létocart *et al.*, 2024). Notably, this reduction in stiffness occurs despite a significant increase in AGE cross-link density in older tendons, suggesting that AGE accumulation alone may not fully explain the mechanical property differences between age groups (Couppe *et al.*, 2009). Given the evident impact of aging on tendon collagen turnover, it is crucial to develop population-specific mitigation strategies, including tailored exercise and nutrition interventions, to preserve tendon health and function in older adults.

### *Sex differences in collagen turnover*

In naturally menstruating, pre-menopausal women, the menstrual cycle is regulated by the production of key hormones, including oestrogen (17-β oestradiol). Serum oestrogen levels are low during menstruation, gradually rise during the late follicular phase (FP) reaching its peak just before ovulation. Following ovulation, oestrogen levels decline during the luteal phase (LP), then rise again to about half their peak value before decreasing once more as the cycle approaches its end (Critchley *et al.*, 2020). Tendons, ligaments and bones contain oestrogen receptors, making them responsive to the female sex hormone (Ciana *et al.*, 2003). Additionally, oestrogen can reduce the expression of LOX in engineered ligaments, which may affect cross-link formation and thus mechanical properties of female tendons (Lee *et al.*, 2015). Therefore, it could be expected that elevated levels of circulating oestrogen, such as those found in healthy, eumenorrheic pre-menopausal women, may impact collagen synthesis and therefore *in vivo* properties of female tendons. Despite this, the *in vivo* evidence in support of these sex differences of this remains limited at present. Cross-sectional work has indicated that water content of female human patellar tendon is greater than that of male tendon, which tentatively suggests this may have implications for tendon mechanical properties (LeMoine *et al.*, 2009) but has not been directly investigated. Furthermore, although LeMone et al. (2009) imply there was a trend for lower collagen content in female tendon, the quantity of collagen and number of cross-links were not significantly different between sexes.

Miller *et al.* (2007) measured the collagen FSR of female patellar tendon at rest and after continuous leg kicking exercise and found female tendon collagen FSR was less than half of that in the males studied in their previous work (Miller *et al.*, 2005a), and there was no change in female tendon collagen FSR 72 h after RE. However, the same

group found an increase in peritendinous PINP at 24 h post-RE in young women, who were not oral contraceptive pill (OCP) users (Hansen *et al.*, 2008; Hansen *et al.*, 2009a). OCPs, commonly combined formulations containing synthetic oestrogen and progesterone, suppress endogenous oestrogen production by inhibiting the hypothalamic-pituitary-gonadal axis, while maintaining steady levels of exogenous oestrogen (Speroff & Fritz, 2005). Interestingly, young women, who were long term users of the combined (oestrogen and progesterone) OCP exhibited lower tendon collagen FSR and PINP (measured in the hours after final pill ingestion) compared with non-OCP users (measured at the onset of menses), which remained unchanged following exercise (Hansen *et al.*, 2009b). While this may reflect an inhibitory effect of synthetic oestrogen and/or progesterone on collagen synthesis, the influence of other hormonal differences between OCP and non-OCP users cannot be ruled out. For example, in addition to exogenous hormone provision, OCP use suppresses endogenous oestrogen, progesterone, luteinising hormone and follicle stimulating hormone resulting in a flat hormonal profile compared to cyclical variations in naturally menstruating non-OCP users (Elliott-Sale *et al.*, 2020). Given the extent to which female sex hormones may influence collagen synthesis, it is perhaps surprising that Miller *et al.* (2007) found no effect of menstrual cycle phase on tendon collagen FSR. A recent case study by Lee *et al.* (2024c) found that higher circulating oestrogen was associated with lower serum PINP after high intensity back squat exercise in a single resistance trained, naturally menstruating female. This case study showed an increase in PINP following RE, however the lower PINP after RE during the late FP (highest circulating oestrogen) compared with the onset of menses (low circulation oestrogen) suggests an inhibiting effect of oestrogen on collagen synthesis**.** The discrepancy with Miller *et al.* (2007) may be due to the difference in exercise intensity

between the study designs (1 h continuous work compared to 4 maximal effort sets). However, it is more likely because Miller *et al.* (2007) collected tissue samples during the early follicular phase and late luteal phase, which were probably not diverse enough in distinguishing circulating oestrogen concentrations to impact collagen synthesis.

Beginning in middle-age, many women experience a peri-menopausal transition involving irregular fluctuations in female sex hormones and menstrual patterns. By their late 40s or early 50s, women usually experience menopause i.e. the complete and permanent cessation of a menstrual period, which is also accompanied by very low circulating oestrogen for the remainder of life. Hansen *et al.* (2009a) observed higher tendon collagen FSR at rest in post-menopausal women using oestrogen replacement therapy (ERT) compared to healthy, age-matched non-users. Despite this, ERT had no effect on PINP in the resting state, nor did exercise increase PINP or tendon collagen FSR in the following days demonstrating a blunted collagen synthesis response to exercise that is not recovered by ERT in postmenopausal women. This discrepancy may be partly explained by lower circulating insulin-like growth factor I (IGF-I) in ERT users, which occurs because oestrogen replacement can suppress growth hormone (GH) production, thereby reducing IGF-I synthesis (Hansen *et al.*, 2013a). As IGF-I plays a key role in stimulating collagen synthesis and tendon adaptation to exercise, its reduction in ERT users may limit the usual anabolic response to exercise despite the increased baseline collagen turnover. The numerous potential interactions of sex hormones on collagen turnover following resistance exercise underscore the importance of researching subgroups of females. Special attention is needed for middle-aged, pre-menopausal, and post-menopausal women, as they are under-

researched, and neglecting these groups could lead to ineffective training and nutrition strategies, compromising their tendon health.

## 2.3 Dietary sources of collagen

***Food and supplemental sources of collagen***

Plant foods do not contain collagen, although some foods such as soybeans and legumes, contain appreciable levels of glycine and proline but lack in other amino acids (AAs), which comprise collagen (Kim *et al.*, 2021; Añazco *et al.*, 2023). As a result, those who voluntarily exclude animal product consumption from their diets are likely to have limited intake compared to omnivores. Dietary collagen protein is exclusive to animal products, and thus omnivores can derive small to moderate amounts of collagen from the consumption of meat products. The amount of collagen can vary greatly between food items, where a small amount of dietary collagen can be consumed from lean cuts of meat (which typically comprise ~ 1 % collagen), moderate amounts of collagen protein from animal muscle containing larger amounts of intramuscular connective tissue (typically ~3% collagen) and even higher intakes can be expect from those eat skin-on animal muscle skin (which comprises ~6% collagen) (FSAI, 2018). Theoretically, very large amounts of collagen can be consumed by eating the tendons, ligaments, and bones of animals. Despite limited data, it is reasonable to suggest that consumption of these tissues is uncommon, although they could be used to make soups, stews and broths. However, the amount of collagen is highly variable and dependant on several factors such as preparation method and source of animal tissue (Alcock *et al.*, 2019a)

Gelatine is a common household product used as a gelling agent in confectionary and other food products such as marshmallows, desserts, and some yogurts. It is extracted from animal collagen by boiling or partial hydrolysis, and is thus comprised of almost 100 % collagen (Alipal *et al.*, 2021). This means that consumption of gelatine-based products could substantially impact collagen intake. Food labelling requirements in the European Union only demand that the quantity of total protein is listed on food labels (EuropeanUnion, 2011, 2016). Therefore, neither the amount of collagen, nor the collagen/protein ratio of meat or gelatine-based products is typically known and requires calculation. Consequently, large observational studies describe the habitual macronutrient intake of various populations but not collagen intake (Sette *et al.*, 2011; Ruiz *et al.*, 2015; Tieland *et al.*, 2015; Hone *et al.*, 2020).

Finally, extracted bovine, porcine, marine and poultry collagen are commonly subjected to enzymatic or acid hydrolysis to produce collagen hydrolysate, a supplemental form of collagen often referred to as collagen peptides, which are powdered and soluble in water (León-López *et al.*, 2019). In 2023, the global collagen market was valued at USD 9.76 billion, with projections indicating a compound annual growth rate (CAGR) of 9.6% from 2024 to 2030 (GrandViewResearch, 2024), indicating that collagen consumption may be increasing at population level through supplementation, although this is currently unknown.

***Tendon nutrition and bioavailability of collagen***

In order for dietary collagen to influence musculoskeletal tissues, it must be digested, absorbed and transported in the circulatory system. The vascularity of tendons is relatively poor compared to other tissues like skeletal muscle (Tempfer & Traweger, 2015). Several human tendons, including the supraspinatus, biceps tendon, Achilles

tendon, and posterior tibial tendon, have either avascular zones, or regions of reduced vascularity (Rathbun & Macnab, 1970; Frey *et al.*, 1990; Ling *et al.*, 1990; Ahmed *et al.*, 1998; Stein *et al.*, 2000). In general, however, the tendon receives most of its blood supply from vessels at the musculotendinous and osseotendinous junctions (Brockis, 1953; Chansky & Iannotti, 1991; Ahmed *et al.*, 1998). In addition, tendons, like other connective tissues, also rely on perfusion from synovial fluid to supply nutrients (Gelberman, 1985; Fenwick *et al.*, 2002), thus the increase in blood flow and synovial fluid following exercise should facilitate synergistic signalling and nutrient delivery to target tissues to enable connective tissue adaptation. Peritendinous blood flow increases approximately 3-fold during exercise (Langberg *et al.*, 1998). Although older (> 70 years) men have lower peritendinous blood flow at rest compared to middle-aged (> 45 years) and younger men, they retain a similar blood flow response following exercise (at least in exercising, healthy Achilles tendons) (Langberg *et al.*, 2001a). Given the limited vascularity of tendons and their reliance on synovial fluid and peritendinous blood flow for nutrient delivery, specific exercise, particularly resistance exercise (RE), appears critical for enhancing nutrient supply and supporting connective tissue adaptation. This is particularly pertinent for ageing tendons, where age-related changes in vascularity may affect tendon metabolism.

Regarding nutrient delivery, it is clear that collagen can be digested, and its constituent AAs absorbed into the circulation. Collagen is notably rich in glycine and proline, as well as being the only dietary source of hydroxyproline (Eastoe, 1955b; Eastoe, 1957). As such, these key AAs have been shown to stimulate collagen synthesis in fibroblasts (Szoka *et al.*, 2017; de Paz-Lugo *et al.*, 2018). Serum concentration of key AAs (e.g., glycine, proline, hydroxyproline) peak ~60 min following collagen ingestion (Alcock *et al.*, 2019b; Alcock *et al.*, 2019c; Lis & Baar, 2019; Skov *et al.*, 2019; Aussieker *et*

*al.*, 2023; Kirmse *et al.*, 2024; Lee *et al.*, 2024c) in a dose-dependent manner (Shaw *et al.*, 2017; Lee *et al.*, 2023c). Interestingly, the bioavailability i.e. timing, peak concentration, and area under the curve (AUC) of key AAs in sera following ingestion appears to be similar regardless of source, given that Alcock *et al.* (2019b) found no difference in these indicators between HC and bone-broth. Similarly, Lis and Baar (2019) found no difference in aminoacidemia or markers of collagen turnover following digestion of HC, gelatine, or a gum mixture comprising both HC and gelatine.

The uptake of collagen AAs following ingestion of collagen, with or without exercise, has yet to be studied in tendon *in vivo.* Despite this, Campbell *et al.* (2023) recently demonstrated that an acute bout of RE results in the delivery of exogenous AAs (bolus including 3 g proline and 2 g glycine) to the peritendinous space of the female Achilles tendon, with those AAs peaking in the microdialysate approximately 90 minutes post-exercise. The high-volume, low intensity exercise protocol and the low dose of exogenous glycine, and proline provided by Campbell *et al.* (2023) may have been insufficient stimuli to increase collagen synthesis, which conflict with those studies using high-intensity exercise, and high doses of collagen intake (Lee *et al.*, 2023c; Lee *et al.*, 2024c). Moreover, the combination of OCP users and non-OCP users in the study by Campbell *et al.* (2023) (three of seven participants used OCP containing ethinylestradiol) likely introduced a high level of variability in responses, despite the authors attempt to account for this in their linear mixed model approach. Finally, Campbell *et al.* (2023) is the only study, at the time of writing, to report higher glycine concentration in peritendinous microdialysate in older compared to younger women following ingestion of AAs with RE. This led the authors to speculate that older tendon is less capable of nutrient uptake, which may confer anabolic resistance, however this

cannot be confirmed by these data alone and warrants further investigation in ageing male and female tendons.

***Habitual dietary intake of collagen***

Alcock *et al.* (2019c) highlight a major challenge in assessing habitual dietary collagen intake as the lack of collagen, gelatine, or AA data available in large databases. Adding to this difficulty, these researchers also found that measuring urinary hydroxyproline (a metabolite of collagen) is not suitable for estimating habitual intake of collagen (Alcock *et al.*, 2019c). Dietary collagen intake can, however, be quantified by estimating the collagen content of food products using analytical sources. Indeed, this is a requirement for food labelling under European Union Legislation, as products labelled as 'meat' are limited to a maximum of 10 % and 25 % collagen content within birds and mammals, respectively, where collagen content derived from analytical methods can be converted to estimates of connective tissue content (EuropeanUnion, 2007; McLean, 2007; TheEuropeanCommission, 2016).

At present, only one study has attempted to describe the habitual dietary intake of collagen at population level. Paul *et al.* (2019) estimated the collagen intake of the standard American diet (SAD) for high consumers of frankfurters, sausages, and luncheon meats (22.6 g collagen/day for males and 12.7 g collagen/day for females), and for those not consuming these highly processed meat-based products (5.3 g collagen/day for males and 3.3 g collagen/day for females). These estimates were calculated by averaging the collagen content (percentage of dry weight) across various food groups and then expressing this as a percentage of the average protein intake for males and females at the population level, as determined by the National Health and Nutrition Examination Survey (NHANES) from 2001-2002 and 2003-2004((CDC),

2002, 2004). There are, however, several limitations with these estimates. Firstly, collagen content estimates were applied to population averages reported by NHANES, and not to individual food diaries contained within the raw data. Secondly, presumably to match the food groups reported by NHANES, the authors averaged the collagen content of multiple food sources together. For example, beef, pork, veal, lamb, and game were given an estimate of 5.15%. This clearly ignores the issue of some food products, even within the same animal, containing vastly different levels of collagen. For example, pork cuts with 100% visual lean content contain only 1.0% collagen, whereas pork rind (less the trimmable fat) contains 22.4% collagen (FSAI, 2018). Finally, these authors did not report what proportion of the American population constituted the so-called high consumers of luncheon meat. It is notable that these authors report sausage meat to comprise approximately 55% collagen, which is more than double the permitted level of collagen in meat products in the EU. This emphasises that caution should be used when extrapolating findings from this study to research participants in other geographical jurisdictions.

## 2.4 Tendon structure and function

### *Tendon structure*

Tendons are comprised mainly of water, however, 60–80% of its dry mass is extracellular matrix (ECM), comprising predominantly type 1 collagen (Kjaer, 2004). The remainder of its dry mass comprises small amounts of collagen III, V, XII and XIV (Thorpe *et al.*, 2013), as well as non-collagenous proteins (a form of glycoproteins known as proteoglycans). These proteoglycans, predominantly decorin and biglycan, are small protein cores covalently attached to glycosaminoglycans

(polysaccharides) (Yoon & Halper, 2005), which play a role in collagen fibrillogenesis, the first step in the biosynthesis of collagen (Vogel & Trotter, 1987).

Like many anatomical structures, tendons are organised in a hierarchical fashion which is essential for their mechanical properties. Collagen forms fibre-like structures at multiple levels, each aligned close to the long axis of the tissue, conferring high levels of uniaxial mechanical strength (Screen *et al.*, 2015). The collagen molecules assemble into fibrils, which bundle into fibres, forming the primary structural units. These fibres group into primary fibre bundles (sub-fascicles), which then form secondary bundles (fascicles), and finally tertiary bundles, providing structural integrity (Benjamin *et al.*, 2008).

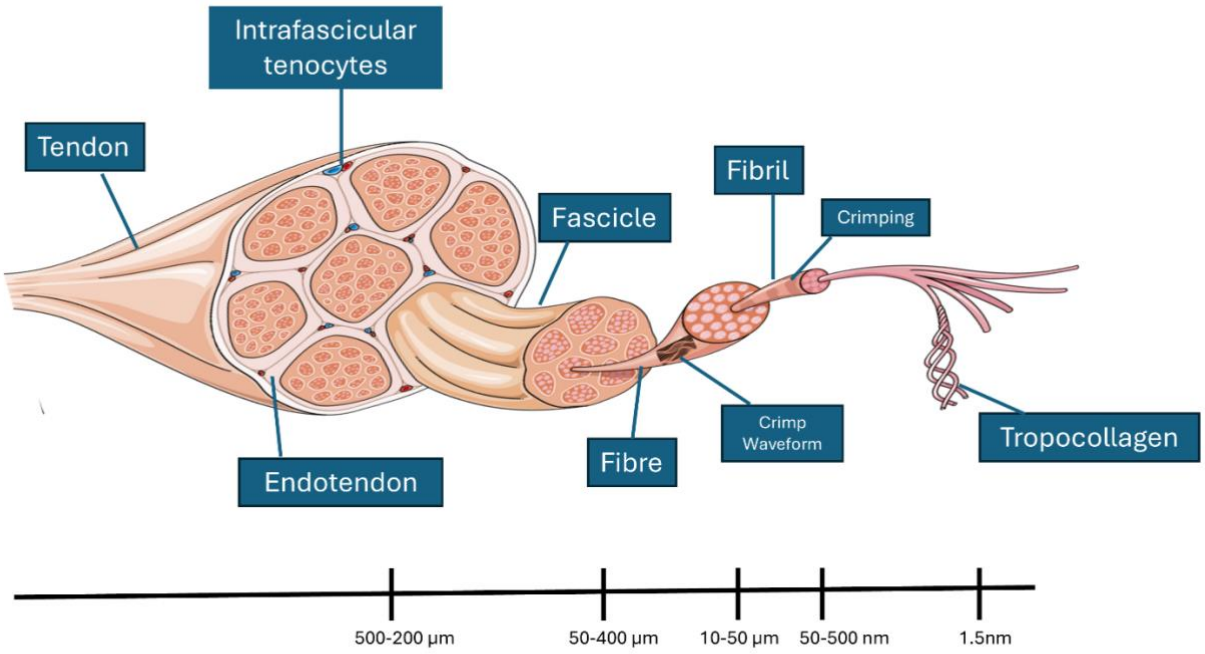


**Figure 1.** Graphical representation of the hierarchical structure of tendon

### *Tendon function*

The parallel alignment of collagen fibres helps resist tension, thereby increasing the efficiency of energy transfer, and limiting contractile energy loss during load transmission (James *et al.*, 2008; Shepherd & Screen, 2013). Consequently, tendon permits force transfer from muscle to bone, causing movement. Prominent lower limb tendons such as the Achilles and patellar tendons transfer force from large muscle groups, and thus experience low *in vivo* loads during activities of daily living, and high loads during athletic movements, especially those involving high velocity and a rapid stretch-shortening, which has implications for athletic performance and injury prevention.

Given the structural and anatomical characteristics of tendon, particularly its stiffness, there is some evidence to suggest tendon properties influence RFD (Bojsen-Møller *et al.*, 2005), since RFD is essentially the rate at which muscle force is transmitted to bone. Young's modulus, a measure of material stiffness, describes how much a tendon resists deformation under load, where a higher modulus indicates a stiffer (less compliant) tendon. Various neuromuscular factors, including maximal strength, muscle size, fibre type, myosin heavy chain isoform composition, and the recruitment and discharge rate of motor units all contribute to RFD (Häkkinen & Komi, 1986; Harridge *et al.*, 1996; Folland *et al.*, 2014; Maffiuletti *et al.*, 2016). However, all these factors being equal, a stiffer tendon should provide greater efficiency of force transfer and ultimately a higher RFD. An increase in tendon compliance, as seen in older adults, would therefore have the opposite effect, i.e. reducing RFD.

### *In vivo* properties of human tendon and adaptations to resistance training

The morphological and mechanical properties of tendon *in vivo* are typically measured by non-invasive medical imaging techniques. Given their superficial location, and their role in major lower limb movement patterns, the Achilles and patellar tendons have been extensively researched. Tendon cross-sectional area (CSA) is typically measured by ultrasonography or magnetic resonance imaging (MRI). Moreover, since human tendon exhibits curvilinear force-elongation and stress-strain relationships (Maganaris & Paul, 1999), the stiffness and Young's modulus (YM) can be measured using synchronised ultrasonography and isokinetic dynamometry (typically during a ramped maximal isometric contraction). There is now a large body of cross-sectional literature that describes the differences in tendon properties between age groups, and between sexes. These techniques have also been used extensively to measure tendon adaptation to repeated mechanical loading i.e. chronic training. Resistance training lasting 6 – 15 weeks can be expected to increase Achilles and patellar tendon stiffness and Young's modulus as well as patellar tendon CSA in young adults (Bohm *et al.*, 2015; Wiesinger *et al.*, 2015a; Lazarczuk *et al.*, 2022) with the majority of tendon hypertrophy occurring at the osteotendinous junctions (Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009).

There is disagreement in the literature regarding the effects of age on tendon CSA, as some studies have shown Achilles and patellar tendons to be similar in size (Carroll *et al.*, 2008; Couppe *et al.*, 2009) or larger in older compared to younger adults (Magnusson *et al.*, 2003b; Couppe *et al.*, 2009; Stenroth *et al.*, 2012; Epro *et al.*, 2017; Couppé *et al.*, 2021; Létocart *et al.*, 2024), which may be the result of habitual loading across the lifespan, or the accumulation of non-contractile material such as AGEs or lipid deposits. In contrast, age-related differences in Achilles and patellar tendon

mechanical properties are consistently reported, as most studies report lower tendon stiffness in older compared to younger (Karamanidis & Arampatzis, 2006; Mian *et al.*, 2007; Carroll *et al.*, 2008; Stenroth *et al.*, 2012; Quinlan *et al.*, 2017; Quinlan *et al.*, 2021; Létocart *et al.*, 2024), although one study found no difference (Couppe *et al.*, 2009). Moreover, two studies demonstrated lower Achilles tendon stiffness in middle-aged, compared to young men (Kubo *et al.*, 2007; Kubo *et al.*, 2022), and one study showed that patellar tendon stiffness is lower in older female tendon compared with middle-aged (Kubo *et al.*, 2003). Furthermore, a single study found lower tendon stiffness in very old (>83 years) vs. old (>65 years) male tendon (Eriksen *et al.*, 2018). The lower tendon stiffness in older humans has also been associated with a lower RFD (Quinlan *et al.*, 2017). While more research is needed to directly assess the relationship between these tendon properties and actual injury risk or performance decline, the observed trends suggest that age-related changes in tendon stiffness could contribute to these outcomes.

The effects of RT on tendon CSA, stiffness, and Young's modulus have also been investigated, given the potential to counteract the age-related decline in tendon properties. Quinlan *et al.* (2021) performed identical concentric and eccentric RT protocols in young and older men and found a smaller increase in patellar tendon stiffness and Young's modulus in the older cohort. These findings are supported by Eriksen *et al.* (2019), who suggested that three months' RT was inadequate time to observe increases in tendon CSA in older adults, with tendon hypertrophy only seen with twelve months' RT. Furthermore, Eriksen *et al.* (2018) found that three months' RT increased muscle strength in old (~68 years) and very old adults (>83 years), however, neither group experienced changes in patellar tendon CSA, and tendon stiffness only improved in the old, but not in the very old group. Another study by

Eriksen *et al.* (2019) found that heavy, not moderate, RT was sufficient to offset the age-related decline in tendon modulus over twelve months. This is in contrast to Létocart *et al.* (2024), who very recently demonstrated that moderate load RT (~55 % one repetition maximum, 1-RM) performed with a slow eccentric contraction improved CSA and stiffness of both Achilles and patellar tendons after 12 weeks. In a similar group of older men only (~67 years), Reeves *et al.* (2003b) observed 65% and 69% increases in patellar tendon stiffness and Young's modulus, respectively, alongside a 27% change in RFD, suggesting the changes in stiffness observed in these studies are likely important for improving physical performance. These studies collectively indicate that moderate- to high-intensity RT can promote beneficial changes in tendon properties, although the degree of adaptation in CSA and stiffness may vary with age, training duration and training intensity. The onset of age-related declines in adaptability to RT remain unclear, since all of the studies comparing young and old, use polarised age groups i.e. adults in their 20s compared to those >65 years to maximise observable differences, yet the adaptability of middle-aged tendon remains unclear.

Sex differences in tendon properties and tendon adaptation have also been documented in the literature. Compared to young men, young women tend to have smaller and more compliant tendons (Magnusson *et al.*, 2007a; Onambélé *et al.*, 2007; Westh *et al.*, 2008; Hicks *et al.*, 2013; Lepley *et al.*, 2018), which may be related to differences in absolute muscle and body size between sexes, or sex differences in collagen turnover (Magnusson *et al.*, 2007a; Miller *et al.*, 2007; Lee *et al.*, 2024c). However, post-menopausal women (not using exogenous oestrogen replacement therapy, ERT) have similar mechanical properties to age-matched men (Burgess *et al.*, 2009) and larger tendon CSA compared to young, pre-menopausal women (Magnusson *et al.*,

2003a; Hansen *et al.*, 2009a). Although this may seem surprising, post-menopausal women (not using ERT) produce low, negligible levels of endogenous oestrogen compared to pre-menopausal women (Burger, 1994; Heshmati *et al.*, 2009), indicating the absence of oestrogen may play a role in differential tendon characteristics.

When investigating the effects of ERT use on tendon properties, Hansen *et al.* (2009a) observed no difference in tendon CSA between post-menopausal users and non-users of ERT. Furthermore, this study found ERT use was associated with lower Young's modulus in post-menopausal women compared to non-ERT users, potentially due to greater density of smaller fibrils (Hansen *et al.*, 2009a).

Some studies, however, have found no difference in tendon properties between groups of females. Svensson *et al.* (2021) found no effect of age or sex on the mechanical properties of human patellar tendon fibrils *ex vivo*, although these data are considered exploratory by the authors, and may not apply to the whole tendon. Three studies found no differences in tendon mechanical properties of young women across different phases of the menstrual cycle, which indicates the effects of oestrogen on whole tendon are likely to be due to chronic, rather than acute exposure (Burgess *et al.*, 2009; Kubo *et al.*, 2009a; Hansen *et al.*, 2013b). Hansen *et al.* (2013b) also compared the tendon properties of long-term OCP users compared to naturally menstruating young women, all of which were handball players, and found no difference between groups.

In relation to RT adaptation, there is evidence to suggest that older female tendons are less responsive compared to older male tendons (Onambele-Pearson & Pearson, 2012). Interestingly, the older females in this study experienced the greatest degree of change in tendon stiffness at low relative force levels (<40% maximum voluntary contraction, MVC), whereas older males experienced greater changes above this level

of relative force. These findings are supported by McMahon *et al.* (2018), who, after 8 weeks' high intensity RT, found no differences in patellar tendon hypertrophy (changes in tendon volume) between young males and females. However, females experienced greater changes at low levels of MVC, whereas males showed greater increases at high MVC force. The reason for these sex-specific adaptations to RT are unclear, but they may be due to lower absolute forces used by females in training, which may evoke specific adaptations. The only RT study which investigated tendinous adaptations in middle-aged women found no effect of 6 months' body weight training on tendon stiffness, most likely due to the low relative intensity of exercise (Kubo *et al.*, 2003), and it is unknown if the use of high intensity RT would yield differential results in this population. Overall, these findings suggest that young women have smaller, more compliant tendons than men, and may experience differential and often lower levels of RT-induced adaptation than young men. Although older women may have more similar tendon properties to older men, their adaptability is still less pronounced.

***Summary***

Tendon morphological and mechanical properties vary based on age and sex, which also influence their adaptability to RT. High intensity RT, eccentric RT and moderate intensity RT (performed with slow controlled tempo) yield similar levels of adaptation to each other. In this regard, young adults generally experience increases in tendon CSA, stiffness, and Young's modulus with RT. Older adults by comparison, exhibit diminished increases in tendon stiffness in the short-term and may require longer RT interventions to experience changes in CSA. There is clear evidence of sex differences in Achilles and patellar tendon properties, with women typically having smaller, more compliant tendons than men. Women potentially exhibit distinct adaptation patterns

e.g., less pronounced changes in stiffness, and greater changes at lower relative forces compared to men, but the evidence for this is limited at present. Collectively, these findings highlight the influence of biological factors of tendon properties and their adaptability to RT, but more work is required to understand middle-aged populations and the mechanisms underpinning sex-specific RT adaptations.

## 2.5 Literature search strategy and table classification

A comprehensive literature search was conducted using academic databases MEDLINE, Web of Science, and Scopus to identify studies investigating the effects of collagen supplementation with exercise on short- and long-term exercise-related outcomes. Search terms combined key words including “collagen”, “gelatin/gelatine”, hydrolysed/hydrolyzed collagen”, “collagen peptides”, “glycine”, “proline”, “hydroxyproline” with terms including “exercise”, “resistance exercise”, “resistance training”, “strength training”, “protein synthesis”, “protein turnover”, “connective tissue”, “collagen synthesis”, “collagen degradation”, “collagen turnover”, “tendon”, “stiffness”, “hypertrophy”, “cross-sectional area”, “strength”, “rate of force development”, and “performance”

The inclusion criteria were broad to capture the diversity in study design, population, and outcome measures. Studies were included if they: (1) were peer-reviewed, human interventions; (2) involved collagen or gelatine supplementation combined with exercise; and (3) assessed at least one relevant outcome: either a short-term outcome such as markers of muscle, tendon, bone turnover, or exercise recovery, or a long-term training adaptation (e.g., changes in macro- or microscopic musculoskeletal properties or exercise performance). Studies were excluded if they were (1) non-human i.e. animal or *in vitro* studies; (2) were not peer-reviewed; (3) did not include collagen or

gelatine in their intervention or (4) lacked outcome measures relevant to musculoskeletal tissue or exercise performance.

Table 1 includes studies examining the acute effects of collagen supplementation with exercise, which were interventions assessing the response to a single bout of exercise, or repeated identical bouts over a short time (≤ 9 days). These studies typically investigated short-term physiological responses, including biomarker changes, amino acid availability, muscle or tendon protein synthesis or markers of recovery from damaging exercise.

Table 2 includes studies investigating the effects of chronic supplementation, i.e. longer-term interventions combining regular collagen supplementation with a structured training programme. These studies primarily describe training-induced physiological adaptations, such as changes in muscle or tendon morphology, strength, or endurance performance.

## 2.6 Effect of acute exercise and collagen ingestion on collagen turnover

A summary of the studies investigating the acute effects of collagen supplementation with exercise is available in Table 1. The study by Shaw *et al.* (2017) examined the dose response of 0, 5, or 15 g collagen (in the form of gelatine), enriched with 48 mg vitamin C following 6 min skipping exercise in healthy young men. In that study, 15 g gelatine ingestion resulted in double the serum procollagen type I N-propeptide (PINP) concentration × time area under the curve (AUC) compared to the 5 g and 0 g gelatine interventions. A follow up study by the same group failed to replicate these findings, however, the authors cited issues with vitamin C potentially

interfering with their PINP assay and increasing between sample variation (Lis and Baar (2019). In a similar design by Hilkens *et al.* (2023), 18 g hydrolysed collagen (HC) with 80 mg vitamin C did not increase serum PINP concentration to a greater extent than 5 min high-impact jumping exercise alone in young men. These discrepancies may be due to subtle differences in exercise stimuli, as jump training alone increased serum peak PINP concentration by ~ 8% in the study by Hilkens *et al.* (2023), whereas skipping alone increased serum PINP concentration by >50 % in the study by (Shaw *et al.*, 2017). Moreover, the discrepancies could be a result of different analytical techniques used, since baseline serum PINP concentration was considerably higher in the study by Hilkens *et al.* (2023) compared with Shaw *et al.* (2017) (~100 ng/mL vs. ~28 ng/mL), despite similar participant characteristics in both studies.

The increases in serum PINP concentration in the skipping and jumping studies likely reflect bone collagen turnover rather than muscle-tendon unit (MTU) turnover, as high-impact activities like these are strong stimulants for bone collagen synthesis (Ng *et al.*, 2023). In contrast, RE is a lower-impact activity that likely stimulates bone collagen turnover to a lesser degree. The effect of RE, a potent stimulus of muscle myofibrillar protein synthesis (Damas *et al.*, 2015) and muscle-tendon collagen synthesis (Miller *et al.*, 2005a), with collagen supplementation has only recently been investigated. Studies have examined the effects of RE with collagen or whey protein ingestion (Oikawa *et al.*, 2020; Aussieker *et al.*, 2023), or RE with a non-caloric placebo (Kirmse *et al.*, 2024) on myofibrillar protein fractional synthetic rate (FSR) and found the effects of collagen to be inferior to whey. This is unsurprising given the discrepancies in amino acid (AA) profile between collagen and whey, especially considering collagen is low in leucine, the key AA known to stimulate muscle protein synthesis, and which is abundant in whey protein (Tang *et al.*, 2009).

Of these studies, two also found no greater effect of 30 g HC ingestion with RE on muscle connective tissue protein FSR in the hours or days after RE (Aussieker *et al.*, 2023; Kirmse *et al.*, 2024), however, several limitations preclude us from drawing conclusive inferences from these data. The use of a between groups design likely introduced a high level of inter-individual variability which may have masked any potential benefits of collagen. Neither of these studies included co-ingestion of vitamin C, which may have limited collagen synthesis (Canty & Kadler, 2005), and in the case of Aussieker *et al.* (2023), the use of a mixed sex cohort may have confounded any results due to potentially lower collagen synthesis in women compared to men (Miller *et al.*, 2007). Moreover, skeletal muscle comprises only a very small proportion (<5%) of collagen, whereas other connective tissues that respond to RE, such as tendon, comprise much higher proportions (~80%). In contrast, Lee *et al.* (2023c) demonstrated that high intensity RE (4 sets of 10-RM back squat) increased the serum PINP concentration × time AUC in the hours after RE, and this was augmented with 30 g, but not 15 g HC ingestion in young, resistance-trained men. Since only the high dose of collagen was effective compared to RE alone, this suggests that a high dose of collagen is likely necessary to augment the acute effects of RE on muscle-tendon collagen synthesis. In a follow-up case study, Lee *et al.* (2024c) found acute RE increased the serum PINP concentration × time AUC in a young, resistance-trained female, and this response was still notable yet diminished in the late follicular phase compared to menstruation, suggesting an inhibitory effect of oestrogen.

Finally, three studies have investigated a blend of whey protein with collagen on acute or short-term exercise responses. One of these studies highlighted that ingesting 5 g collagen alongside 25 g whey overcomes the post RE decline in plasma glycine in young, healthy men (Aussieker *et al.*, 2024a). In a follow up study using the same

blend, Aussieker *et al.* (2024b) found that this combination of collagen and whey stimulated both myofibrillar and muscle connective tissue synthesis postprandially in the rested leg, but not the exercised leg. This directly conflicts with their previous work, where neither 30 g whey or collagen impacted muscle connective tissue synthesis rates (Aussieker *et al.*, 2023). The lack of a 100 % whey or collagen in comparison to the collagen/whey blend in (Aussieker *et al.*, 2024b) prevents the identification of which nutrients (whey or collagen) impacted muscle connective tissue synthesis. Furthermore, the discrepancies between (Aussieker *et al.*, 2023) and (Aussieker *et al.*, 2024b) may underscore the limitation of assessing muscle collagen protein turnover following collagen ingestion, given the inherently low collagen content of skeletal muscle.

Another study by Robberechts *et al.* (2023) compared daily supplementation with 25 g whey + 20 g HC or 45 g whey protein in parallel groups of young men during three weeks of damaging exercise, but reported no differences between groups with regards to markers of muscle damage. The authors claim this study was designed to investigate the effect of collagen supplementation on markers of muscle damage; however, the absence of an isocaloric or non-caloric placebo group limits its ability to address this question. This limitation is further compounded by the high protein doses provided to both groups. While the authors attempted to match supplements for protein content, it is impossible to isolate the effects of different nutrients since both groups received at least 25 g of whey protein which has previously been shown to attenuate markers of exercise-induced muscle damage (Buckley *et al.*, 2010; Eddens *et al.*, 2017; Brown *et al.*, 2018). Additionally, Rindom *et al.* (2016), using a crossover design, reported similar levels of muscle strength and anaerobic exercise performance recovery in the 48 hours following high intensity RE regardless of whether young men supplemented

with 25 g whey or 25 g collagen. Moreover, ingestion of 15 g or 20 g HC has been shown to attenuate declines in countermovement jump performance following damaging exercise in young resistance trained men compared to placebo (Clifford *et al.*, 2019; Prowting *et al.*, 2020). Overall, while the combination of whey and collagen may have some potential to support muscle connective tissue synthesis and mitigate muscle damage, these effects remain uncertain and further research is needed to clarify the underlying mechanisms and resolve existing inconsistencies.

***Summary***

Studies on the acute effects of collagen supplementation combined with exercise suggest that musculoskeletal responses depend on dose, exercise intensity and participant characteristics. Collagen's amino acid profile, notably its high glycine, proline, and hydroxyproline content, may positively influence connective tissues, as shown by augmented serum PINP responses, although these effects are inconsistent. While collagen supplementation has limited impact on myofibrillar protein FSR due to its amino acid profile, it may benefit connective tissues, such as tendons and ligaments, particularly with high-intensity RE. Additionally, collagen supplementation with high-impact exercises may positively influence bone turnover and help mitigate muscle damage. However, most studies have been conducted in young, active, predominantly male populations, and further research is needed to optimize collagen supplementation strategies across diverse populations.

**Table 1.** Summary of studies investigating the acute effects of collagen supplementation with exercise

| Study | Design | Participants | Exercise Type | Supplementation | Key Outcome Measures | Key Findings |
|---|---|---|---|---|---|---|
| Shaw et al. (2017) | Randomised, double-blind, crossover | 8 healthy, recreationally active young men (27 ± 6 y, 79.6 ± 12 kg) | 6 min rope-skipping | 5 g or 15 g vitamin C-enriched gelatine, placebo control | Serum amino acids (glycine, proline, hydroxyproline); collagen synthesis (PINP) | - 15 g gelatine increased serum PINP ~2× compared to placebo and 5 g. |
| Clifford et al. (2019) | Double-blind, independent groups | 24 recreationally active males (CP: 24.1 ± 4.3 y, 79.6 ± 7.5 kg, 177.8 ± 3.8 cm; CON: 24.8 ± 4.8 y, 76.1 ± 10.9 kg, 180.2 ± 6.7 cm) | 150 drop jumps | 20 g/day collagen peptides or placebo for 7 days before and 2 days after exercise | Muscle soreness, countermovement jump (CMJ) height, β-CTX, PINP, inflammation, muscle damage, pressure pain threshold, mood | - Muscle soreness was lower in the collagen group at 48 h post-exercise (large effect size: ES = 2.64).<br>- CMJ height recovered faster in the collagen group at 48 h (P = 0.050; ES = 0.55).<br>- No effect on inflammation or bone collagen turnover markers (β-CTX, PINP). |

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| Lis and Baar (2019) | Randomised, double-blind, crossover | 10 recreationally active males (22.7 ± 5.2 y, 179.0 ± 8.0 cm, 78.8 ± 7.7 kg, 13.6 ± 3.1% body fat) | 6 min rope-skipping | 15 g vitamin C-enriched gelatine, hydrolysed collagen (HC), gummy with equal parts gelatine & HC, placebo | N-terminal propeptide of procollagen (PINP), serum amino acids | - Gelatine and HC increased PINP by ~20% from baseline.<br>- No significant difference between the supplements and placebo.<br>- All supplements increased circulating amino acids similarly.<br>- Variability in results prevented statistical significance for any treatment. |
| Prowting et al. (2019) | Randomised, double-blind, independent groups | 15 resistance-trained males (CP: 7 participants, placebo: 8 participants) | 5 sets of 20 drop jumps | 15 g/day collagen peptides or placebo for 7 days | Maximal voluntary isometric contraction (MVIC), countermovement jump (CMJ) height, muscle soreness, collagen turnover biomarkers | - CP supplementation attenuated CMJ height decline at 24 hr post-exercise.<br>- Muscle soreness was significantly higher than PRE at 24 hr and 48 hr in both groups.<br>- MVIC showed a significant time effect but no changes in collagen biomarkers.<br>- Acute CP consumption provided a performance benefit 24 hr post-damaging exercise. |

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| Oikawa et al. (2020) | Double-blind, randomised, crossover | 11 endurance-trained participants (5 male, 6 female, 24 ± 4 y, VO2 max = 53.2 ± 9.1 mL·kg·min) | 4 × 4-min cycling intervals at 70% peak power for 3 consecutive days | 20 g α-lactalbumin (LA) or collagen peptides (CP), 40 g before sleep | Myofibrillar protein synthesis (MyoPS), sarcoplasmic protein synthesis (SarcPS), plasma leucine, tryptophan | - LA ingestion increased plasma leucine and tryptophan significantly ($P < 0.001$) compared to CP.<br>- Both supplements increased protein synthesis, with LA showing greater gains in MyoPS (13%) and SarcPS (5%) than CP ($P < 0.01$). |
| Oikawa et al. (2020b) | Double-blind, parallel-group design | 22 healthy older women (69 ± 3 y, n = 11/group) | Unilateral resistance exercise (RE) twice during 6 days | 30 g whey protein (WP) or collagen protein (CP) twice daily for 6 days | Acute and long-term muscle protein synthesis (MPS), [$^{13}C_6$]-phenylalanine infusion, deuterated water | - WP increased MPS at rest ($P < 0.01$), CP did not<br>- MPS increased to greater extend after exercise in WP compared to CP ($P = 0.02$).<br>- Long-term MPS was significantly greater with WP than CP in both Rest ($P < 0.001$) and Exercise ($P < 0.001$). |
| Centner et al. (2022) | Randomised, double-blind, parallel | 30 recreationally active young | High-load leg extension exercise | 15g hydrolysed collagen (COL) or placebo (PLA) post-exercise | Gene expression in pathways related to skeletal muscle | COL: Greater upregulation of PI3K-Akt and MAPK pathways at 4 hours post-exercise compared to PLA. No significant differences in mTOR pathway. |

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| | | men (25.4 ± 2.4 years) | (single session) | | signal transduction (PI3K-Akt, MAPK, mTOR) | |
| Hilkens et al. 2023 | Randomised, cross-over | 14 healthy males (24 ± 4 y, BMI 22.0 ± 2.1 kg/m²) | 5 min high-impact exercise | 20 g hydrolysed collagen or placebo | Serum PINP, CTX-I (fasted & postprandial) | PINP: No significant change with collagen vs. placebo (P = 0.58)<br>CTX-I: No significant difference (P = 0.17)<br>Fasted PINP increased ~8% with daily exercise (P < 0.01) |
| Aussieker et al. (2024a) | Randomised, double-blind, crossover | 14 recreationally active men (26 ± 5 y, BMI: 23.8 ± 2.1 kg·m²) | RE | Various blends: Whey protein + 0 g (WHEY), 5 g (WC05), 10 g (WC10), or 15 g (WC15) collagen | Plasma amino acids (glycine, leucine, essential, nonessential) | Plasma glycine increased with WC05, WC10, WC15 (P < 0.05); No differences in total amino acid availability between treatments (P > 0.05); Plasma leucine and essential amino acids higher in WHEY than WC10 and WC15 (P < 0.05); Co-ingestion of 5 g collagen with whey protein prevents glycine decline during recovery |
| Aussieker et al. (2024b) | Randomised, double-blind, parallel | 28 recreationally active young men (25 ± 5 y; | Unilateral resistance exercise (leg press | 30 g whey + collagen protein blend (25 g whey, | Plasma amino acids, myofibrillar protein synthesis | - BLEND increased plasma leucine and glycine concentrations (P < 0.001).- MyoPS rates were higher in BLEND vs. PLA in both rested (P < 0.05) and exercised (P < |

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| | | BMI 23.6 ± 2.3 kg/m²) | and leg extension, 5 sets at 80% 1RM) | 5 g collagen) or placebo | (MyoPS), muscle connective protein synthesis | 0.01) legs.- Muscle connective protein synthesis rates were higher in BLEND vs. PLA in the rested leg (P < 0.05), but not the exercised leg (P = 0.11). |
| Aussieker et al. (2023) | Randomised, double-blind, parallel | 45 recreationally active young men and women (25 ± 4 y; BMI 24.1 ± 2.0 kg/m²) | 6 sets barbell back squats at 60 % 1-RM | 30 g whey, 30 g collagen, or placebo | Plasma amino acids, myofibrillar protein synthesis (MyoPS), muscle connective protein synthesis | - WHEY increased MyoPS rates vs. PLA (P < 0.05), but COLL did not.- Muscle connective protein synthesis rates did not differ significantly between groups (P = 0.09).- WHEY increased leucine/essential amino acids; COLL increased glycine/proline (P < 0.05). |
| Robberechts et al. (2023) | Randomised, double-blind, parallel | 22 fit young men (W: 24.4 ± 2.4 y; WCP: 20.8 ± 1.9 y; BMI: 22.7 ± 1.4 kg/m²) | 3-week eccentric training: unilateral knee extensions (5-8 sets, 20 reps at | 45 g/day whey or 25 g whey + 20 g collagen peptides | Muscle damage markers (creatine kinase, serum PINP), functional performance (CMJ, MVIC) | - Both groups showed transient impairments in MVIC (~10%) and increased creatine kinase after training (P < 0.01).- Training improved CMJ (+8%) and MVIC (+10%) in both groups (P < 0.01).- No differences between whey and whey + collagen peptide groups for any outcome. |

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| | | | 70–75% 25-RM), one-leg squats, and drop jumps (40–60 cm) | | | |
| Lee et al. (2023) | Randomised, double-blind, crossover | 10 resistance-trained males (26 ± 3 y; BMI: ~25.5 kg/m²) | 4 sets barbell back squats at 10-RM | 0 g, 15 g, or 30 g hydrolysed collagen (HC) + 50 mg vitamin C | Serum PINP, β-CTX, glycine, proline (AUCs) | - PINP AUC was higher for 30 g HC than 15 g and 0 g HC ($P < 0.05$).- Glycine and proline AUCs were greater for 30 g HC than for 15 g and 0 g HC ($P < 0.05$).- No differences in β-CTX between interventions. |
| Lee et al. (2024) | Randomised, double-blind, crossover | 1 female athlete (36 y; 1.61 m; 82.6 kg) | 4 sets barbell back squats at 10-RM | 0 g or 30 g hydrolysed collagen (HC) | Serum 17β-oestradiol, PINP, β-CTX, collagen amino acids | - PINP AUC was higher for 30 g HC during low oestrogen (OM) phase (201 $\mu g \cdot L^{-1} \cdot h$) vs. high oestrogen (LF) (144 $\mu g \cdot L^{-1} \cdot h$).- β-CTX decreased in all interventions.- High oestrogen was associated with lower collagen synthesis after RE.- 30 g HC |

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| | | | | | | augmented collagen synthesis more during low oestrogen. |
| Kirmse et al. (2024) | Randomised, double-blind, parallel design | 25 young men (24 ± 3 y; 76.9 ± 6.4 kg) | 1 week resistance exercise | 15 g hydrolysed collagen peptides (2× daily) or placebo | Plasma glycine, proline, hydroxyproline, muscle protein synthesis rates (myofibrillar and connective) | - Collagen peptides increased plasma glycine, proline, and hydroxyproline ($P < 0.05$).- No differences in myofibrillar or connective protein synthesis rates between collagen and placebo groups ($P > 0.05$).- Collagen peptides did not enhance muscle protein synthesis during intense training. |
| McKendry et al. (2024) | Randomised controlled trial, double-blind | 31 older males (72 ± 4 years) | No exercise intervention | Whey, pea, or collagen protein supplementation (50 g/day) above recommended daily allowance of protein (RDA, 0.8 g/kg/day) | Integrated myofibrillar protein synthesis (MPS), anabolic signalling (mTORC1, rpS6), postprandial aminoacidemia | - MPS increased with whey (1.59 ± 0.11 %/d) and pea (1.59 ± 0.14 %/d) protein, compared to RDA (1.46 ± 0.09 %/d for whey and 1.46 ± 0.10 %/d for pea).- No increase in MPS with collagen supplementation.- Supplemental whey and pea, but not collagen protein enhanced anabolic signalling and postprandial aminoacidemia at rest. |

## 2.7 Effect of chronic resistance training with collagen ingestion on muscle-tendon unit properties

A summary of the studies investigating the effects of collagen supplementation with long-term exercise is available in Table 2. In the last decade, several studies have investigated the effects of combined chronic RT and collagen supplementation, with earlier work focussing on muscle strength and fat-free mass, rather than tendon. The earliest study reported that 12-weeks' RT with 15 g HC increased fat free mass (FFM) by 4.2 kg and decreased fat mass (FM) by 5.5 kg in older, sarcopenic men measured by dual x-ray absorptiometry (DXA) (Zdzieblik *et al.*, 2015). This study has received criticism for its remarkable findings (Phillips *et al.*, 2016), which have not been replicated. Despite this, some additional studies have suggested that collagen supplementation with RT has positive effects of fat-free mass compared with placebo, however, it is difficult to draw strong conclusions given these findings rely on data derived from bio-electrical impedance analysis (BIA) (Jendricke *et al.*, 2019; Kirmse *et al.*, 2019a; Oertzen-Hagemann *et al.*, 2019). BIA is known to be sensitive to temperature and hydration status of participants (Baumgartner *et al.*, 1990); relies on several assumptions and regression equations to estimate FM and FFM, and the limbs have been known to disproportionately contribute to whole body impedance (Coppini *et al.*, 2005). DXA is currently considered the 'gold standard' to assess changes in FM and FFM, although this method is also affected by hydration status (Toomey *et al.*, 2017)). Skinfold measurements, while less precise than DXA, offer a more reliable alternative to BIA for assessing body composition, as they are less affected by the factors influencing BIA, although they do rely on a high level of practitioner skill/experience (Kasper *et al.*, 2021).

Additionally, RT with collagen supplementation does not augment muscle fibre CSA compared to RT alone (Kirmse *et al.*, 2019a), while other studies have reported inferior changes in muscle fibre CSA, muscle thickness and lean body mass (measured with DXA) when RT was supplemented with 20 – 35 g collagen rather than 20 – 35 g whey protein (Wageh *et al.*, 2021; Jacinto *et al.*, 2022b; Wageh *et al.*, 2024). The lack of effect of collagen supplementation on RT induced gains in muscle size is predictable, as collagen supplementation has been shown not to influence myofibrillar FSR (Oikawa *et al.*, 2020; Aussieker *et al.*, 2023; Kirmse *et al.*, 2024). In contrast, the only study to find a positive effect of collagen supplementation with RT on muscle hypertrophy reported greater increases in vastus medialis muscle volume, but not in the other three heads of the quadriceps muscle (Balshaw *et al.*, 2022), which is unusual. None of these studies measured changes in passive connective tissues such as tendon or ligament where collagen is more abundant than skeletal muscle. Furthermore, tendon and ligament are likely more sensitive to exogenous collagen, given it contains very high levels of glycine and proline compared to whey, and is the only protein to contain hydroxyproline, all of which stimulate collagen synthesis *in vitro* (Surazynski *et al.*, 2010; de Paz-Lugo *et al.*, 2018).

The small number of studies that have measured the effects of RT with HC supplementation on the tendon properties in young men and women generally show positive findings. Jerger and colleagues demonstrated that 5 g HC ingested daily during 14-week period of high intensity (70 – 85% 1-RM) RT increased Achilles (Jerger *et al.*, 2022) and patellar (Jerger *et al.*, 2023) tendon CSA, although there were no additional increases in tendon stiffness beyond the effects of RT alone in either study. This apparent uncoupling of gains in size and stiffness is unusual considering the notably large gains (+11%) in the CSA of both tendons with collagen

supplementation compared to placebo (5-7%). This may be due to how tendon stiffness was measured, as both studies calculated stiffness from the slope of the force-elongation curve at 50–80% pre-training MVC. While this approach controls for stiffness increases resulting purely from increased force, and is generally considered a strength, it may be considered a limitation in untrained participants. The disproportionately large strength gains observed in untrained individuals, compared to those expected in trained participants, likely shifted some post-training stiffness measurements into the 'toe' region of the force-elongation curve. This may have introduced variability in the stiffness data, potentially obscuring any effects of supplementation, in contrast to the more uniform strength and hypertrophy results.

In academy level female soccer athletes, however, Lee *et al.* (2023a) found that 30 g HC and 500 mg vitamin C with bodyweight and plyometric training increased patellar tendon stiffness and Young's modulus by ~18 % compared to ~5 % with training alone after 10 weeks. Similarly, in professional female soccer athletes performing high-intensity RT, these authors found greater gains in patellar tendon stiffness and modulus in the cohort ingesting 30 g HC compared to the placebo group (Lee *et al.*, 2024a). Notably, the incorporation of once-weekly high intensity RE was sufficient to increase tendon CSA to a small extent, regardless of supplementation. In contrast to Jerger and colleagues' (2022; 2023) findings in young men, the lack of effect of HC on tendon hypertrophy in the studies by Lee and colleagues (2023a; 2024a) are likely due to the lower intensity RE in the academy athletes, and limited frequency (only once per week) of high intensity RE sessions in the professional players. Collectively, these studies show that HC supplementation enhances RT-induced changes in tendon properties, however the specificity of these effects appears to depend on the exercise intensity and frequency, athletic status (including training history and sex), and dosage

of supplementation. In contrast, one study by Balshaw *et al.* (2023) found no effect of 15 weeks' RT with 15 g daily HC supplementation on patellar tendon CSA, stiffness, or Young's modulus. The reason for the discrepancy between this Balshaw *et al.* (2023) and others is unclear, however, this may be explained by the small effects of RT alone in the untrained men i.e. an increase in PT CSA of 1.7 %, which was not statistically significant, and much lower than expected increase in PT CSA of ~ 5 % (Wiesinger *et al.*, 2015a).

Two studies have found a positive effect of RT with HC supplementation on changes in knee extensor RFD (Lis *et al.*, 2021; Bischof *et al.*, 2023). Three weeks of high intensity RT with plyometrics and speed training led to an overall decrease in RFD, measured during an isometric squat in male collegiate athletes. However, RFD decreased less in the group consuming 20 g HC with vitamin C compared to the placebo group (Lis *et al.*, 2021). Although this suggests some benefit of collagen supplementation for lower body explosive exercise performance, the short intervention duration was likely insufficient to fully evaluate its effects. Additionally, the authors noted that training was highly variable and not standardized across participant subgroups, with many failing to complete the prescribed progression scheme. These significant limitations make it challenging to draw definitive conclusions about the efficacy of the intervention. Bischof *et al.* (2023) conducted a longer-term intervention, measuring the ability of previously untrained males to recover from an acute bout of damaging exercise before and after 12 weeks of concurrent RT and endurance training with or without 15 g of daily collagen supplementation. In this study, RFD during isometric knee extension recovered similarly between groups prior to the training intervention; however, the collagen group recovered RFD quicker than the placebo group post-12-week intervention.

Conversely, in the study by Balshaw *et al.* (2022), the authors state that HC did not increase explosive strength to a greater extent than RT alone. However, the study reported significant increases at specific time points (100 and 150 ms after force onset for HC and placebo groups, respectively), indicating the peak explosive force (and thus peak RFD) may have occurred at different time points for each group, therefore some between group differences may have been overlooked. Indeed, there was a tendency for a group × time interaction regarding explosive force at 50 ms after force onset (P = 0.054), with HC increasing by 11 % and PLA not changing (-2%, independent t-test $d = 0.51$). However, as part of the same study, the authors stated that total quadriceps volume was augmented more in HC than PLA despite no group × time interaction (P = 0.076), leading to their claim that just one of the four quadriceps heads increased its volume more in HC than in PLA, with no between group differences in hypertrophy regarding the other three muscles. None of these studies simultaneously measured the properties of the lower limb tendons, but it can be speculated that greater changes to the tendon material properties in those supplementing with HC offered a protective effect against reductions in RFD associated with damaging and fatiguing exercise, given the positive correlation between tendon stiffness and RFD (Bojsen-Møller *et al.*, 2005; Quinlan *et al.*, 2017). This hypothesis cannot be confirmed from existing data, as no study to date has shown concomitant improvements in tendon properties and RFD following RT with collagen supplementation. Overall, HC with RT results in neutral to positive effects on gains in fat-free mass and strength in untrained and older individuals, yet has little impact on muscle hypertrophy. HC has the greatest effect on RT induced gains in tendon size and stiffness in young healthy populations and may confer additional benefits during explosive movements, although this combination has yet to be investigated.

**Table 2.** Summary of studies investing the effects of chronic resistance exercise combined with collagen supplementation on changes in musculoskeletal properties

| Study | Participants | Exercise Type | Supplementation | Outcome Measures | Key Findings |
|---|---|---|---|---|---|
| Zdzieblik et al. (2015) | 53 elderly men with sarcopenia (72.2 ± 4.7 years) | Full-body resistance training (3x/week, 12 weeks) | 15g hydrolysed collagen (COL) or placebo (PLA) daily | Fat-free mass (FFM), fat mass (FM), body mass (BM), isokinetic knee extension strength (KE), sensory motor control | COL: Greater ↑ FFM, KE, BM; ↓ FM compared to PLA |
| Oertzen-Hagemann et al. (2019) | 25 young men (24.2 ± 2.6 years) | Full-body resistance training (3x/week, 12 weeks) | 15g hydrolysed collagen (COL) or placebo (PLA) daily | BM, FFM, FM, strength, skeletal muscle proteome | RT ↑ BM, FFM, strength (COL > PLA); COL: ↑ 221 proteins linked to contractile fibres vs. ↑ 41 in PLA |
| Jendricke et al. (2020) | 59 recreationally active women (25.4 ± 4.2 years) | Concurrent training (3x/week, 12 weeks) | 15g hydrolysed collagen (COL) or placebo (PLA) daily | Running distance, velocity and heart rate at lactate and anaerobic thresholds, body composition (FM, FFM), muscular strength (1RM), muscular endurance | COL: Greater ↑ in running distance, FFM, and reduction in heart rate at anaerobic threshold compared to PLA. Both groups improved in velocity at thresholds, FM, 1RM, and muscular endurance with no significant |

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| | | | | | differences between groups |
| Zdzieblik et al. (2021) | 97 middle-aged, untrained men (51.8 ± 4.56 years) | Full-body resistance training (3x/week, 12 weeks) | 15g hydrolysed collagen (COL), placebo (PLA), or whey protein (WHEY) daily | FFM, FM, body weight (BW), waist circumference (WC), isometric knee extension strength | COL: Greater ↑ FFM, ↓ FM compared to PLA; WHEY also improved FFM but not significantly different from PLA |
| Mertz et al. (2021) | 208 healthy older adults (>65 years) | Light-intensity resistance training (LITW) 3-5x/week or heavy resistance training (HRTW) 3x/week for 1 year | 20g hydrolysed collagen (COL), 20g whey protein (WHEY), or carbohydrate (CARB) twice daily | Quadriceps cross-sectional area (qCSA), dynamic peak torque (DPT), maximal voluntary isometric contraction (MVIC), rate of force development (RFD), leg extensor power (LEP), 400m gait time, body composition | COL and WHEY did not significantly affect any measured parameter compared to CARB. HRTW improved qCSA, DPT, and MVIC compared to WHEY. LITW increased DPT compared to WHEY. |
| Wageh et al. (2021) | 26 healthy young adults (22 ± 2 years) | Linear progressive resistance | 20g whey protein, 2g leucine, 2.5g creatine monohydrate, 300mg | LBM, FM, BF%, BMC, BMD, muscle thickness and CSA (vastus lateralis, | WHEY: Greater ↑ in LBM, biceps brachii CSA and |

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| | | training (4x/week, 10 weeks) | calcium citrate, 1000 IU vitamin D (WHEY) or 20g hydrolysed collagen (COL), 1.4g alanine, 0.6g glycine (COL) twice daily | biceps brachii), 1RM (leg press, bench press, lat pull-down), isometric unilateral torque, muscle fibre type distribution and CSA | thickness, type II fibre CSA compared to COL. Both groups showed significant increases in strength measures with no significant differences between groups. |
| Jerger et al. (2022) | 40 healthy men (26.3 ± 4.0 years) | High-load resistance training (3x/week, 14 weeks) | 5g hydrolysed collagen (COL) or placebo (PLA) daily | Achilles tendon cross-sectional area (CSA), tendon stiffness, muscle thickness, maximal voluntary torque | COL: Greater ↑ in tendon CSA and muscle thickness compared to PLA. Both groups improved tendon stiffness and muscle strength with no significant difference between groups. |
| Jacinto et al. (2022) | 22 healthy untrained young adults | Supervised resistance training (3x/week, 10 weeks) | 35g whey protein (WHEY) or leucine-matched collagen peptides (COL) daily | Muscle thickness (MT) of vastus lateralis and biceps brachii, isokinetic peak torque, mean power output of elbow flexors, peak power output of lower body | WHEY: Greater ↑ in vastus lateralis and biceps brachii MT compared to COL. Both groups showed |

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| | | | | | similar increases in muscle performance (peak torque, mean power output, and peak power output). |
| Balshaw et al. (2022) | 39 young healthy men (27.0 ± 5.0 years) | Lower-body resistance training (3x/week, 15 weeks) | 15g hydrolysed collagen (CP) or placebo (PLA) daily | Knee extensor and flexor isometric strength, quadriceps, hamstrings, and gluteus maximus volume (MRI), evoked twitch contractions, 1RM lifting strength, muscle architecture (ultrasound) | CP: Greater ↑ in vastus medialis (VM) volume, twitch peak torque, and angle of pennation of the VM compared to PLA. No significant differences in maximum strength (isometric or 1RM) between groups. |
| Balshaw et al. (2023) | 39 young healthy men (27.0 ± 5.0 years) | Lower-body resistance training (3x/week, 15 weeks) | 15g hydrolysed collagen (CP) or placebo (PLA) daily | Patellar tendon cross-sectional area (CSA), vastus lateralis (VL) aponeurosis area, patellar tendon stiffness, Young's modulus, tendon elongation, strain | No change in CSA in either group. No difference between CP and PLA groups in any tendinous tissue adaptations. Both |

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| | | | | | groups showed increases in VL aponeurosis area, patellar tendon stiffness, and Young's modulus, and decreases in tendon elongation and strain. |
| Jerger et al. (2023) | 50 healthy, moderately active men (28.6 ± 5.1 years) | High-load resistance training (3x/week, 14 weeks) | 5g hydrolysed collagen (COL) or placebo (PLA) daily | Patellar tendon cross-sectional area (CSA), tendon stiffness, maximal voluntary knee extension strength, rectus femoris muscle CSA | COL: Greater ↑ in patellar tendon CSA compared to PLA. Both groups improved tendon stiffness, muscle CSA, and muscular strength with no significant difference between groups. |
| Jerger et al. (2023) | 32 moderately trained men (28.4 ± 2.4 years) | Concurrent training (60 min moderate intensity running + 15 min dynamic resistance training, | 15g hydrolysed collagen (COL) or placebo (PLA) daily | Running distance in a 1-hour time trial, velocity at lactate threshold (V*LT), velocity at individual anaerobic threshold (V*IAT), body composition | COL: Greater ↑ in running distance, V*LT, and V*IAT compared to PLA. Both groups showed similar reductions in fat |

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| | | 3x/week, 12 weeks) | | | mass and increases in fat-free mass. |
| Bischof et al. (2023) | 55 predominantly sedentary men (26.1 ± 5.1 years) | Concurrent training (30 min resistance + 30 min endurance, 3x/week, 12 weeks) | 15g hydrolysed collagen (COL) or placebo (PLA) daily | Maximum voluntary contraction (MVC), rate of force development (RFD), peak RFD, countermovement jump height (CMJ), muscle soreness (MS), body composition | COL: Faster recovery in MVC, RFD, peak RFD, and CMJ height compared to PLA. No significant differences in muscle soreness and body composition between groups. |
| Lee et al. (2023) | 17 female soccer players (17 ± 0.9 years) | Soccer training (bodyweight strength, plyometric, and pitch-based exercises, 3x/week, 10 weeks) | 30g hydrolysed collagen (COL) or placebo (PLA) daily | Patellar tendon (PT) stiffness, Young's modulus, tendon cross-sectional area (CSA), knee extension (KE) maximum isometric voluntary contraction (MIVC) torque, vastus lateralis muscle thickness | COL: Greater ↑ in PT stiffness and Young's modulus compared to PLA. No significant changes in tendon CSA, KE MIVC torque, or muscle thickness in either group. |
| Wageh et al. (2024) | Randomised, double-blind, parallel design | 26 participants (males and females) | 10-week linear resistance training (RT) | Multi-ingredient supplement (MIS: whey protein, creatine, leucine, calcium citrate, vitamin D) or collagen (COL) | Satellite cell (SC) content, myonuclear accretion, SC and myonuclear |

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| | | | | | domain, fiber cross-sectional area (fCSA) |
| Lee et al. (2024) | 11 professional female soccer athletes (25.7 ± 4.2 years) | Pre-season soccer training (high-intensity resistance, plyometric, and pitch-based exercises, 3x/week, 10 weeks) | 30g hydrolysed collagen (COL) or placebo (PLA) daily | Patellar tendon (PT) stiffness, Young's modulus, tendon cross-sectional area (CSA), knee extension (KE) maximum isometric voluntary contraction (MIVC) torque, vastus lateralis muscle thickness | COL: Greater ↑ in PT stiffness and Young's modulus compared to PLA. Both groups showed a significant increase in PT CSA, but no significant differences between groups. No significant changes in KE MIVC torque or muscle thickness in either group. |

## 2.8 Summary and conclusion

Collagen is a crucial protein that constitutes approximately 30% of total protein in the human body, and type I collagen provides structural integrity to musculoskeletal tissues. Specifically, tendons comprise primarily type I collagen and play a vital role in force transmission from muscle to bone. A single bout of resistance exercise acutely increases whole body markers of collagen synthesis, which probably plays a crucial role in augmenting tendon cross-sectional area, stiffness and elastic modulus when performed repeatedly over weeks to months. Ingestion of collagen supplements prior to a single bout of resistance exercise augments markers of whole-body collagen synthesis but not myofibrillar and muscle connective tissue protein synthesis young men and women. These observations likely explain why changes in tendon size and stiffness are augmented after 8-12 weeks' resistance training in young female athletes and young men, while changes in muscle size and strength are largely unaffected by collagen supplementation. This review also highlights that while collagen ingestion leads to the appearance of collagen amino acids in the blood, indicating greater bioavailability, there is virtually no evidence of the quantity of collagen consumed in an adult habitual diet. Thus, it is not clear whether certain demographics have insufficient collagen intake, and whether they would benefit from collagen supplementation to augment the bioavailability of key amino acids to increase collagen synthesis and potentially improve tendon health and performance. The enhancements in tendon properties with RT and collagen supplementation are likely to have implications for explosive exercise performance and injury risk, and despite some short-term studies showing that RFD may recover faster with collagen supplementation, the long term-effects of collagen with RT on RFD are yet to be fully

elucidated. Furthermore, ageing has been associated lower circulating IGF-I (Nielsen *et al.*, 2014), increased AGE accumulation (Couppe *et al.*, 2009) and increased collagen degradation (Kragstrup *et al.*, 2011), all of which may impair collagen remodelling. Hormonal factors also appear to play a role in tendon remodelling. Although the role of endogenous oestrogen (and other hormones) in regulating collagen turnover is not fully understood, key interactions likely exist and may vary based on hormonal status (Hansen *et al.*, 2009a; Lee *et al.*, 2024c). For example, the use of oral contraceptives (containing ethinyl oestradiol and progestins) has been associated with a blunted tendon collagen synthesis response to exercise (Hansen *et al.*, 2009b). Thus, both age and sex are likely to influence the rate and magnitude of RT-induced changes in tendon properties. While studies have shown positive (yet less pronounced) effects of RT on tendon properties in older adults, no studies have investigated the effects of RT with collagen supplementation on changes in tendon properties in middle-aged/ older men or women.

# Chapter Three

# Habitual dietary collagen intake is lower in women and older Irish adults compared to younger men

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## Prelude

Collagen, the most abundant protein in connective tissues, plays a critical role in skin, bone, and tendon health. Despite its importance, very little is known about dietary collagen intake at population level. in fact, the habitual dietary collagen intake of European adults is currently unknown. Using the latest available data from the Irish National Adult Nutrition Survey (NANS), this chapter will provide the first comprehensive estimate of habitual collagen intake in a nationally representative sample ($n$ = 1,500) of male and female Irish adults, aged 18 – 39 years (young), 40 – 64 years (middle-aged), and ≥65 years (older adults). Information from this study may be used to inform changes in dietary behaviour (e.g. the need for collagen supplementation in certain demographics).

# Abstract

Collagen ingestion is purported to benefit connective tissues, such as skin, bone, muscle, tendon, and ligament. However, the quantity of collagen intake in the diet of European adults is unknown. The aim of this study was to investigate collagen intake in the habitual diets of Irish adults, and whether it differed according to sex and/or age. We conducted secondary analysis of the Irish National Adult Nutrition Survey, which assessed typical dietary intake using a four-day food diary in 1,500 adults, aged 18-90 years. We categorized participants into three age groups: young (18-39 years, n = 630), middle-aged (40-64 years, n = 644), and older (≥ 65 years, n = 226) adults. Collagen composition of each individual food item in the database was determined by applying a percentage collagen value from analytical sources, allowing computation of collagen mean daily intake (MDI), collagen MDI relative to body mass, and collagen/total protein MDI. Differences in intakes between age groups and sexes were evaluated using physical activity level as a covariate. Collagen MDI for the entire population was 3.2±2.0 $g \cdot day^{-1}$, representing 3.6±1.9% total protein intake. Men had higher absolute and relative collagen MDI than women, regardless of age (4.0 ± 2.1 $g \cdot day^{-1}$ vs. 2.3 ± 1.4 $g \cdot day^{-1}$, $p < 0.001$), while older adults had lower absolute collagen MDI than middle-aged adults (2.9 ± 1.8 $g \cdot day^{-1}$ vs. 3.3 ± 2.0$g \cdot day^{-1}$, $p = 0.021$). Collagen intake in the Irish adult population was considered low (relative to total protein intake and to dose-response studies), particularly in women and older individuals. Increasing daily collagen intake may therefore be warranted to optimise the health of collagen-rich tissues.

# 3.1 Introduction

Collagen is the most abundant protein in the human body, found in the extracellular matrix (ECM) of connective tissue (e.g., in skin, bones, tendon, ligament and cartilage). Collagen is synthesized by fibroblasts through a process that involves the transcription of collagen genes, translation into pre-procollagen, and extensive post-translational modifications, with vitamin C being a crucial co-factor for proper folding into a triple-helix structure (Canty & Kadler, 2005). Collagen synthesis decreases with age, while collagen degradation increases simultaneously, leading to reduced dermal volume and elasticity, which can be observed as increased skin wrinkles and slower cutaneous wound healing (Jun & Lau, 2010; Calleja-Agius *et al.*, 2013). Moreover, collagen loss with age is associated with bone loss and increased tendon compliance, which are both risk factors for higher fracture rates and fall injuries in older adults (Reeves *et al.*, 2006; Tomlinson *et al.*, 2019).

Dietary collagen is rich in glycine, proline, and hydroxyproline, with the latter being exclusive to collagen-containing foods. While collagen can be synthesized endogenously, dietary intake is required to maintain sufficiently high levels of these amino acids, particularly glycine, which may be conditionally essential in humans (Meléndez-Hevia *et al.*, 2009; Li & Wu, 2018). For ingested collagen to contribute to systemic collagen turnover, it must undergo enzymatic hydrolysis in the gastrointestinal tract, being digested into peptides and free amino acids prior to being absorbed into the blood stream (Virgilio *et al.*, 2024). In recent years, there has been significant interest on the effects of collagen intake, with and without exercise, on skin appearance, wound healing, joint pain, exercise recovery, body composition, sleep quality, and muscle and tendon function (Miyab *et al.*, 2020; Khatri *et al.*, 2021;

Holwerda & van Loon, 2022; Thomas *et al.*, 2024). Medium to long-term supplementation with 2.5 to 12 g hydrolysed collagen (HC) appears to have positive effects on skin ageing by increasing elasticity and hydration, and reducing the appearance of wrinkles in adult women aged 20 – 70 years (de Miranda *et al.*, 2021). Additionally, collagen ingestion combined with acute skipping or resistance exercise has been associated with increased markers of collagen synthesis, with doses ranging between 5 and 30 g collagen in young healthy, recreationally trained men and women (Shaw *et al.*, 2017; Lee *et al.*, 2023c; Lee *et al.*, 2024c). Moreover, 15 to 30 g daily HC supplementation in conjunction with chronic exercise is purported to improve fat-free mass, tendon morphology, and markers of strength in both athletic and untrained adults (Bischof *et al.*, 2024).

These findings indicate positive effects of increasing dietary collagen intake, however, there is no established recommended daily allowance for collagen, and we cannot assume the dietary collagen requirements of adults are the same across the age range and between sexes. Firstly, this is because ageing is associated with collagen loss, especially after 40 years of age (Varani *et al.*, 2006; Baumann, 2007). Secondly, connective tissues comprising mostly collagen, such as ligament, tendon and bone, all contain estrogen receptors (Liu *et al.*, 1997; Hart *et al.*, 1998; Lee & Lanyon, 2004), resulting in sex-specific collagen turnover (Miller *et al.*, 2007). Thus, before making recommendations on increasing collagen intake (either via dietary food or supplementation), the amount of collagen ingested in the habitual diets of both men and women across the age range must be documented.

Despite the clear interest in collagen supplements in areas of dermatology, musculoskeletal health, ageing, and sports performance, only one study, to our knowledge, has attempted to estimate collagen intake in habitual diets. Paul *et al.*

(2019) estimated that the average daily collagen protein consumption in the 'standard American diet' to be either 3 or 23 g·day$^{-1}$ (based on whether individuals were low or high consumers of processed meat), which was derived from the National Health and Nutrition Examination Survey (NHANES) 2001-2004. However, estimates of collagen in food items were unjustifiably averaged across a limited number of food items (e.g., beef, pork, veal, lamb, and game), which likely confounded the estimations of collagen intake, thus potentially providing erroneous conclusions.

The National Adult Nutrition Survey (NANS) investigated habitual food and beverage consumption in 1,500 adults in Ireland between 2008 and 2010. By conducting secondary analysis of NANS, we determined collagen composition of each individual food item in the database by applying a percentage collagen value from analytical sources, allowing computation of collagen mean daily intake (MDI), relative MDI (g·kg$^{-1}$), and collagen/total protein MDI (%). We categorized participants into young, middle-aged and older adults, and we compared men with women. Thus, the objective of our study was to determine the habitual dietary collagen intake of Irish adults, stratified by age group and sex, using detailed collagen content data from specific food items. In addition, due to vitamin C being an essential component for collagen synthesis (Canty & Kadler, 2005), we investigated vitamin C intake according to age and sex. Finally, as increased physical activity is associated with a higher metabolic rate and greater energy intake (Beaulieu *et al.*, 2016), we used habitual physical activity as a covariate in our dietary analyses. Additionally, habitual physical activity (PA) is positively associated with protein intake in older adults across multiple populations (Lourida *et al.*, 2021), suggesting a potential link between PA levels and collagen intake, given that collagen is a specific source of dietary protein. Based on the higher habitual protein intake in younger men versus older men and women (Hone

*et al.*, 2020), we hypothesized that absolute and relative collagen MDI would be lower in older and middle-aged individuals compared to young adults, and lower in women compared to men.

## 3.2 Methods

### Population

The current study is a secondary analysis of a cross-sectional food consumption survey among Irish adults (described in detail elsewhere (Hone *et al.*, 2020)). The original survey was the Irish NANS, conducted by the Irish Universities Nutrition Alliance (IUNA; www.iuna.net). This survey included 1,500 free-living adults aged 18–90 years (740 men and 760 women) residing in the Republic of Ireland between 2008 and 2010. Ethical approval was granted by the University College Cork Clinical Research Ethics Committee of the Cork Teaching Hospitals and the Human Ethics Research Committee of University College Dublin. All participants provided written consent in line with the *Declaration of Helsinki*. Participants were randomly chosen from a database of names and addresses provided by Data Ireland (National Postal Service). An invitation letter and participant information sheet were sent to the homes of potential participants. Participants were excluded if they were pregnant or lactating or were unable to complete the survey due to disability. The survey achieved a response rate of 59.6 %, and the final sample was demographically representative of the Irish population in terms of sex, age, location, social class, and geographic distribution according to the 2006 Irish census. The sample size has previously been demonstrated to be sufficient for detecting dietary intake differences by sex and age in recent secondary analyses of this dataset (Hone *et al.*, 2020).

**Dietary assessment**

A four-day food diary (detailed at the product brand level where possible) was employed to record food, beverage, and supplement intake. Participants were required to include at least one weekend day in their recordings. Researchers visited participants' homes three times during the four-day period: the first visit demonstrated how to use a food weighing scales and maintain the food diary; the second visit, 24-36 hours into the recording process, reviewed the diary entries; and the final visit, 1-2 days after the recording period, reviewed the last entries and collected the diary. Food and beverage consumption was quantified using food weighing scales where possible. However, for items that were not weighed, portion sizes were estimated using a photographic food atlas, a food portion size guide, household measurements, manufacturer weights, the IUNA weight guide, and researcher estimates. Nutrient intakes were estimated using WISP, version 3.0 (Tinuviel Software, Anglesey, UK), based on data from McCance and Widdowson's "The Composition of Foods," 5th and 6th editions and their associated supplementary volumes (Holland *et al.*, 1988; Holland *et al.*, 1989; McCance & Widdowson, 2014). Dietary intake was averaged across the four days to provide mean daily intakes (MDI) for all nutrients of interest.

**Calculation of collagen composition**

The collagen content of food items was determined by applying a percentage weighting from estimated typical values. A database (SPSS v. 29, IBM, Armonk, NY, USA) was created containing all of the 2552 NANS foods consumed, including recipes. Secondly, this database was examined on a food code-by-food code basis, and each food code was assigned a collagen concentration based on analytical data and other published data sources, which are presented in Table 1 (McBride *et al.*, 1960;

Olley, 1981; Nuckles *et al.*, 1990; Stevenson *et al.*, 1992; El, 1995; Sivakumar *et al.*, 1997; Chemistry, 2002; Combes *et al.*, 2004; Park *et al.*, 2007; Danos *et al.*, 2008; Nishimura *et al.*, 2011; FSAI, 2018; Teixeira *et al.*, 2019; Daszkiewicz & Janiszewski, 2020). In total, 736 foods were identified to contain collagen. If a food item contained a meat mixture, i.e. more than one meat cut or meat source, then the total collagen for that item was calculated using the following equation, which was modifiable to include additional ingredients as required (FSAI, 2018):

$$\frac{(A\times a)+(B\times b)+(C\times c)\ldots}{100}$$

where A, B, C, etc., represent the percentage of each meat cut present, and a, b, c, etc., represent the percentage of collagen in each meat cut. For example, an item containing 15% beef brisket lean (2.56% collagen), and 3% beef fat (5.76% collagen) would be calculated as:

$$\frac{(15\ \times\ 2.56) + (3\ \times\ 5.76)}{100} = \frac{38.4 + 17.28}{100}$$

$$= 0.56\%\ collagen$$

Collagen values for foods that were prepared whole but contain inedible portions such as bone were then multiplied by their edible conversion factor from the composition of foods integrated dataset (COFID) (PublicHealthEngland, 2021). For database items where the food label was not available, or the item was a meal/multi-ingredient item, e.g. beef lasagna, or chicken korma, the percentage of foods containing collagen was determined by dividing the mass (g) of each respective food containing collagen in the recipe by the sum of the mass (g) of all items in the recipe expressed as a percentage of total mass. Where mixed foods were sandwiches without precise quantities of the

constituents, the recipe from the University of London survey of commercial sandwiches was used for all calculations (University of London School of Life Sciences, 1997). Finally, where collagen content of meat was presented as a percentage of total protein content, rather than a percentage of total weight (e.g. anchovies, bovine liver), the known total protein was multiplied by the collagen percentage to determine the collagen value for that food item.

**Table 1**. Typical percentage values for total protein, collagen, and fat in meat and animal derived food products. *VL* refers to the percentage visual lean content of meat.

| Meat Cut | Protein (%) | Collagen (%) | Connective Tissue (%) | Fat (%) | Literature Source |
|---|---|---|---|---|---|
| **Pork** | | | | | |
| Lean – top quality 100VL | 19.0 | 1.00 | 5.3 | 8.9 | FSAI (2008) |
| Lean containing a small amount of visible fat and connective tissue 95VL | 18.0 | 1.40 | 7.8 | 13.8 | FSAI (2008) |
| Lean containing no major gristles 90VL | 16.5 | 2.00 | 12.1 | 19.3 | FSAI (2008) |
| Sow lean 80VL | 18.0 | 2.30 | 12.8 | 26.4 | FSAI (2008) |
| Lean trimmings (incl. hock) 80VL | 17.0 | 3.40 | 20.0 | 27.4 | FSAI (2008) |
| Lean with fat 50VL | 11.5 | 1.90 | 16.5 | 53.6 | FSAI (2008) |
| More fat than lean 40VL | 6.5 | 1.10 | 16.9 | 61.8 | FSAI (2008) |
| Coarse fatty tissue containing a little lean | 5.0 | 1.50 | 1.50 | 76.5 | FSAI (2008) |
| Pork Neck Lean | 18.6 | 1.92 | 10.3 | 11.5 | FSAI (2008) |
| Pork Neck 85VL | 16.6 | 2.24 | 13.5 | 22.2 | FSAI (2008) |
| Pork Neck 85VL including Rind | 17.4 | 3.12 | 17.9 | 21.1 | FSAI (2008) |
| Pork Hand Joint Lean | 19.4 | 2.08 | 10.7 | 8.8 | FSAI (2008) |
| Pork Hand 90VL | 17.9 | 2.64 | 14.8 | 16.8 | FSAI (2008) |
| Pork Hand 90VL including Rind | 18.9 | 3.84 | 20.3 | 16.0 | FSAI (2008) |
| Pork Loin Lean | 20.9 | 1.68 | 8.0 | 8.4 | FSAI (2008) |

| | | | | | |
|---|---|---|---|---|---|
| Pork Loin 85VL | 17.4 | 2.08 | 12.0 | 23.9 | FSAI (2008) |
| Pork Loin 85VL including Rind | 18.9 | 3.76 | 19.9 | 22.5 | FSAI (2008) |
| Pork Belly Lean | 19.8 | 1.84 | 9.3 | 9.9 | FSAI (2008) |
| Pork Belly 80VL | 16.4 | 2.48 | 15.1 | 25.5 | FSAI (2008) |
| Pork Belly 80VL including Rind | 17.8 | 4.16 | 23.4 | 23.8 | FSAI (2008) |
| Pork Leg Lean | 20.7 | 1.6 | 7.7 | 5.0 | FSAI (2008) |
| Pork Leg 95VL | 18.8 | 2.00 | 10.6 | 13.8 | FSAI (2008) |
| Pork Leg 95VL including Rind | 19.4 | 2.96 | 15.2 | 14.0 | FSAI (2008) |
| Pork 95VL Desinewed | 17.3 | 0.55 | 3.2 | 12.0 | FSAI (2008) |
| Back Fat | 5.1 | 3.68 | 71.8 | 78.6 | FSAI (2008) |
| Flare Fat | 3.0 | 1.80 | 60.0 | 82.6 | FSAI (2008) |
| Semi-lean rind on | 16.0 | 3.20 | 20.0 | 48.6 | FSAI (2008) |
| Rind with fat uncooked (35% fat) | 22.0 | 14.20 | 64.5 | 35.0 | FSAI (2008) |
| Rind less trimmable fat uncooked (10% fat) | 34.5 | 22.40 | 64.9 | 10.0 | FSAI (2008) |
| Rind with fat cooked (derived from A12) | 17.0 | 11.00 | 64.7 | - | FSAI (2008) |
| Gristle | 22.0 | 14.20 | 64.5 | - | FSAI (2008) |
| Masseter Muscle | 20.0 | 3.90 | 19.5 | - | FSAI (2008) |
| Diaphragm | 15.0 | 10.60 | 70.7 | - | FSAI (2008) |
| Rehydrated Drinde 95VL | 22.0 | 14.20 | 64.5 | - | FSAI (2008) |
| **Beef** | | | | | |
| Lean – top quality 100VL | 21.0 | 1.50 | 7.1 | 8.7 | FSAI (2008) |

| | | | | | |
|---|---|---|---|---|---|
| Lean containing a small amount of visible fat and connective tissue 95VL | 20.0 | 3.00 | 15.0 | 12.6 | FSAI (2008) |
| Lean with a moderate amount of visible fat and connective tissue 85VL | 17.0 | 3.40 | 20.0 | 22.3 | FSAI (2008) |
| Lean with some fat 75VL | 16.0 | 4.80 | 30.0 | 30.6 | FSAI (2008) |
| More fat than lean 30VL | 10.0 | 3.00 | 30.0 | 72.5 | FSAI (2008) |
| Beef Brisket Lean | 16.3 | 2.56 | 15.7 | 27.6 | FSAI (2008) |
| Beef Brisket 75VL | 15.2 | 2.88 | 19.0 | 32.4 | FSAI (2008) |
| Beef Jacob’s Ladder Lean | 18.6 | 2.40 | 12.9 | 18.4 | FSAI (2008) |
| Beef Jacob’s Ladder 85VL | 17.8 | 2.48 | 14.0 | 22.1 | FSAI (2008) |
| Beef Fore Rib Lean | 18.3 | 2.16 | 11.8 | 20.9 | FSAI (2008) |
| Beef Fore Rib 80VL | 17.1 | 2.24 | 13.1 | 25.9 | FSAI (2008) |
| Beef Chuck Lean | 19.4 | 2.48 | 12.8 | 13.1 | FSAI (2008) |
| Beef Chuck 95VL | 18.9 | 2.64 | 14.0 | 15.8 | FSAI (2008) |
| Beef Thin Flank Lean | 18.4 | 2.32 | 12.6 | 21.1 | FSAI (2008) |
| Beef Thin Flank 80VL | 16.6 | 2.64 | 15.9 | 28.8 | FSAI (2008) |
| Beef Shin and Leg Lean | 21.8 | 3.92 | 18.0 | 6.2 | FSAI (2008) |
| Beef Shin and Leg | 21.4 | 4.72 | 22.0 | 9.9 | FSAI (2008) |
| Beef Clod and Sticking Lean | 19.2 | 2.96 | 15.4 | 14.7 | FSAI (2008) |
| Beef Clod and Sticking 90VL | 18.5 | 3.20 | 17.3 | 18.2 | FSAI (2008) |
| Beef Topside Lean | 21.8 | 1.60 | 7.4 | 6.3 | FSAI (2008) |

| | | | | | |
|---|---|---|---|---|---|
| Beef Topside 95VL | 20.6 | 1.84 | 8.9 | 11.6 | FSAI (2008) |
| Beef Loin Rump and Fillet Lean | 19.6 | 2.00 | 10.2 | 14.8 | FSAI (2008) |
| Beef Loin Rump and Fillet 85VL | 18.0 | 2.16 | 12.0 | 22.2 | FSAI (2008) |
| Beef Thick Flank and Silverside Lean | 20.6 | 2.48 | 12.1 | 9.6 | FSAI (2008) |
| **Poultry** | | | | | FSAI (2008) |
| Skinless Chicken Breast | 23.7 | 0.62 | 2.6 | 2.1 | FSAI (2008) |
| Skinless Chicken Leg | 19.9 | 1.84 | 9.3 | 5.2 | FSAI (2008) |
| Skinless Chicken Thigh | 19.7 | 1.12 | 5.7 | 7.1 | FSAI (2008) |
| Skinless Mixed Chicken Meat | 19.4 | 1.68 | 8.6 | 7.7 | FSAI (2008) |
| Chicken Breast with Skin | 22.1 | 1.2 | 5.4 | 6.7 | FSAI (2008) |
| Chicken Leg with Skin | 18.8 | 2.4 | 12.8 | 10.1 | FSAI (2008) |
| Chicken Thigh with Skin | 17.2 | 1.84 | 10.7 | 12.9 | FSAI (2008) |
| Mixed Chicken Meat with Skin | 16.1 | 3.44 | 21.4 | 23.2 | FSAI (2008) |
| Chicken Ground Desinewed (Fronts) | 17.1 | 0.69 | 4 | 15.6 | FSAI (2008) |
| Chicken Fat | 3 | 2 | 66.7 | - | FSAI (2008) |
| Chicken Skin | 11.8 | 5.68 | 48.3 | 44.2 | FSAI (2008) |
| Skinless Turkey Breast | 23.9 | 0.64 | 2.7 | 2 | FSAI (2008) |
| Skinless Turkey Leg | 19.6 | 1.44 | 7.4 | 6 | FSAI (2008) |
| Skinless Turkey Thigh | 19.8 | 1.12 | 5.7 | 5.7 | FSAI (2008) |
| Skinless Mixed Turkey Meat | 22.1 | 1.6 | 7.2 | 6.5 | FSAI (2008) |
| Turkey Breast with Skin | 23 | 1.04 | 4.5 | 5.4 | FSAI (2008) |

| | | | | | |
|---|---|---|---|---|---|
| Turkey Leg with Skin | 18.9 | 1.92 | 10.1 | 9.7 | FSAI (2008) |
| Turkey Thigh with Skin | 19.1 | 1.68 | 8.8 | 10.3 | FSAI (2008) |
| Mixed Turkey Meat with Skin | 18.4 | 2.88 | 15.6 | 17.9 | FSAI (2008) |
| Turkey Skin | 12.3 | 6.56 | 53.6 | 49.4 | FSAI (2008) |
| **Marine** | | | | | |
| Pollock (Meat) | 21 | 1.2 | 4.5 | 2.5 | Kim et al. (2007) |
| Pollock (Skin) | 19.5 | 57.1 | 5 | 1 | Kim et al. (2007) |
| Herring (Meat) | 18 | 2.5 | 4 | 3 | McBride et al. (1960) |
| Herring (Skin) | 20 | 50 | 5 | 2 | McBride et al. (1960) |
| Sardine (Meat) | 21.8 | 1.36 | - | 2.2 | Sato et al. (1986) |
| Rainbow Trout (Meat) | 21 | 1.88 | - | 0.8 | Sato et al. (1986) |
| Sea Bass (Meat) | 21.6 | 3.52 | - | 0.1 | Sato et al. (1986) |
| Japanese Eel (Meat) | 16.1 | 7.96 | - | 15.6 | Sato et al. (1986) |
| Smooth Dogshark (Meat) | 27.9 | 8.48 | - | - | Sato et al. (1986) |
| Spiny Dogfish (Meat) | 22.9 | 4.52 | - | - | Sato et al. (1986) |
| Red Stingray (Meat) | 24.6 | 3.76 | - | - | Sato et al. (1986) |
| Conger Eel (Meat) | 18.7 | 8.76 | - | - | Sato et al. (1986) |
| Pike Conger (Meat) | 20.6 | 6.16 | - | - | Sato et al. (1986) |
| Sweetfish (Meat) | 18.8 | 3.56 | - | - | Sato et al. (1986) |

| | | | | | |
|---|---|---|---|---|---|
| Brook Masu Salmon (Meat) | 19.9 | 1.68 | - | - | Sato et al. (1986) |
| Argentine (Meat) | 20.3 | 1.72 | - | - | Sato et al. (1986) |
| Carp (Meat) | 18.9 | 2.4 | - | - | Sato et al. (1986) |
| Striped Mullet (Meat) | 22.3 | 4.64 | - | - | Sato et al. (1986) |
| Horse Mackerel (Meat) | 21.9 | 2.04 | - | - | Sato et al. (1986) |
| Nibbler (Meat) | 21 | 3.52 | - | - | Sato et al. (1986) |
| Red Sea Bream (Meat) | 24.9 | 2.92 | - | - | Sato et al. (1986) |
| Chub Mackerel (Meat) | 23.7 | 2 | - | - | Sato et al. (1986) |
| Devil Stinger (Meat) | 20.7 | 2.72 | - | - | Sato et al. (1986) |
| Bastard Halibut (Eye Side) | 22.4 | 5.56 | - | - | Sato et al. (1986) |
| Bastard Halibut (Blind Side) | 22.6 | 5.48 | - | - | Sato et al. (1986) |
| Mud Dab (Meat) | 24.5 | 4.32 | - | - | Sato et al. (1986) |
| Black Scraper (Meat) | 19.3 | 2.72 | - | - | Sato et al. (1986) |
| Painted Comber (Meat) | 20.3 | 0.41 | - | - | Sikorski et al. (1984) |
| Rockfish (Meat) | 21.1 | 0.64 | - | - | Sikorski et al. (1984) |
| Snapper (Meat) | 21.3 | 0.56 | - | - | Sikorski et al. (1984) |
| Bigeyed Sea Perch (Meat) | 18.7 | 0.45 | - | - | Sikorski et al. (1984) |
| Red Bream (Meat) | 18.8 | 0.36 | - | - | Sikorski et al. (1984) |
| Bermuda Catfish (Meat) | 19.1 | 0.4 | - | - | Sikorski et al. (1984) |
| Marbled Notothenia (Meat) | 16.2 | - | - | - | Sikorski et al. (1984) |
| Navaga (Meat) | 19 | 0.4 | - | - | Sikorski et al. (1984) |

| | | | | | |
|---|---|---|---|---|---|
| Plaice (Meat) | 13.6 | 0.3 | - | - | Sikorski et al. (1984) |
| Rock Sole (Meat) | 14.4 | 0.43 | - | - | Sikorski et al. (1984) |
| Flathead Sole (Meat) | 15.6 | 0.3 | - | - | Sikorski et al. (1984) |
| Yellowfin Sole (Meat) | 15 | 0.28 | - | - | Sikorski et al. (1984) |
| Brown Sole (Meat) | 14.4 | 0.37 | - | - | Sikorski et al. (1984) |
| Australian Pilchard (Meat) | 16.5 | 0.29 | - | - | Sikorski et al. (1984) |
| Round Scad (Meat) | 17.5 | 0.18 | - | - | Sikorski et al. (1984) |
| Atlantic Bonito (Meat) | 20.6 | 0.66 | - | - | Sikorski et al. (1984) |
| Dolphin (Meat) | 15.6 | 0.5 | - | - | Sikorski et al. (1984) |
| Yellowfin Tuna (Meat) | 18.7 | 0.51 | - | - | Sikorski et al. (1984) |
| Bigeye Tuna (Meat) | 19.1 | 0.52 | - | - | Sikorski et al. (1984) |
| Spiney Lantern Shark (Meat) | 16.6 | 0.79 | - | - | Sikorski et al. (1984) |
| Squid, Loligo vulgaris (Mantle) | - | 2.68 | - | - | Sikorski et al. (1984) |
| Squid, Todarodes pacificus (Skin) | - | 2.68 | - | - | Sikorski et al. (1984) |
| Octopus, Octopus vulgaris (Arm) | - | 1.1 | - | - | Sikorski et al. (1984) |
| Octopus, Octopus vulgaris (Skin) | - | 1.9 | - | - | Sikorski et al. (1984) |
| Abalone, Haliotis discus (Adductor Muscle) | - | 0.4 | - | - | Sikorski et al. (1984) |
| Abalone, Haliotis discus (Foot Muscle) | - | 0.8 | - | - | Sikorski et al. (1984) |
| Prawn, Penaeus indicus (Muscle) | 20.9 | 0.5 | - | - | Sivakumar et al. (1996) |

| | | | | | |
|---|---|---|---|---|---|
| **Offal (based on fattener pigs)** | | | | | |
| Liver | 25.12 | 0.91 | - | 3.51 | Babicz et al. (2023) |
| Heart | 17.41 | 2.16 | - | 6.39 | Babicz et al. (2023) |
| Kidney | 16.48 | 1.83 | - | 4.9 | Babicz et al. (2023) |
| **Paté** | | | | | |
| Goat + 10% pork belly | 18.9 | 1.34 | - | 9.7 | Teixeira et al. (2019) |
| Goat + 30% pork belly | 18.7 | 1.4 | - | 13.6 | Teixeira et al. (2019) |
| Goat + 10% olive oil | 19.2 | 1.54 | - | 11.4 | Teixeira et al. (2019) |
| Goat + 30% olive oil | 19.6 | 1.54 | - | 16.6 | Teixeira et al. (2019) |
| Sheep + 10% pork belly | 22.3 | 1.3 | - | 14 | Teixeira et al. (2019) |
| Sheep + 30% pork belly | 21.9 | 1.19 | - | 18.2 | Teixeira et al. (2019) |
| Sheep + 10% olive oil | 20.7 | 1.54 | - | 13.4 | Teixeira et al. (2019) |
| Sheep + 30% olive oil | 21 | 1.46 | - | 18 | Teixeira et al. (2019) |
| **Game** | | | | | |
| Rabbit loin | - | - | - | 1.7 | Comes et al. (2004) |
| Pheasant (Pectoralis major) | 25.13 | 0.2 | - | 0.19 | Daszkiewicz and Janiszewski (2020) |
| **Lamb and Mutton** | | | | | |
| Lean Lamb of the Leg and Chump | 19.4 | 1.68 | 8.7 | 10.1 | FSAI (2008) |
| Lean Lamb of the Loin and Best End Neck | 18.4 | 1.76 | 9.5 | 18 | FSAI (2008) |

| | | | | | |
|---|---|---|---|---|---|
| Lean Lamb of the Scrag Shoulder Middle Neck and Breast | 17.1 | 1.92 | 11.2 | 21.3 | FSAI (2008) |
| 90VL Leg and Chump | 17.9 | 1.92 | 10.7 | 17.8 | FSAI (2008) |
| 80VL Loin and Best End Neck | 16 | 1.84 | 11.5 | 29.8 | FSAI (2008) |
| 80VL Scrag Shoulder Middle Neck and Breast | 15.9 | 2 | 12.6 | 27.4 | FSAI (2008) |
| Lean Fore-quarter Mutton | 16.7 | 2 | 12 | 23.1 | FSAI (2008) |
| Lean Hind-quarter Mutton | 19.3 | 1.76 | 9.1 | 11.6 | FSAI (2008) |
| 80VL Fore-quarter Mutton | 15.4 | 2.08 | 13.5 | 29.1 | FSAI (2008) |
| 90VL Hind-quarter Mutton | 17.7 | 1.92 | 10.9 | 19.6 | FSAI (2008) |

**Physical activity levels**

Participants completed a validated physical activity questionnaire (EPIC Physical Activity Questionnaire (EPAQ2)) (Wareham *et al.*, 2002) to estimate habitual levels of physical activity. The questionnaire comprised three sections: activity at (i) home, (ii) work and (iii) recreation. To estimate participants' metabolic equivalent of the task (MET) values, we used the EPAQ2 responses to calculate the average MET hours spent per week. MET values were assigned to various activities based on established MET values for each type of activity. The total weekly MET hours were then calculated by summing the MET hours from all three activity domains (home, work, and recreation). Participants were subsequently categorized into three activity levels based on their total weekly MET hours (Bayles, 2023): low activity: < 7.5 MET hours per week; moderate activity: ≥ 7.5 to 15 MET hours per week; high activity: > 15 MET hours per week.

**Secondary data analysis**

Data were analysed using the Statistical Package for the Social Sciences (SPSS v. 29, IBM, Armonk, NY, USA) and reported as mean ± standard deviation, with significance accepted at $P < 0.05$. The following new variables were computed and used for the analysis of collagen intake: absolute collagen mean daily intake (MDI) in grams; relative collagen MDI in g/kg (collagen MDI relative to body mass); and collagen/total protein MDI (%) (collagen MDI expressed as a percentage of total protein MDI).

To examine the effects of age-group and sex on collagen intake, participants were assigned to one of two categories for sex (male or female), and one of three categories for age: (i) young (18–39 years, male, n = 331; female, n = 299); (ii) middle-aged (40–64 years, male, n = 303; female, n = 341); and (iii) older (≥ 65 years, male, n = 106;

female, n = 120). Data were assessed for normal distribution using visual inspection of Q-Q plots. Most nutrient intake variables approximated normality despite slight tail deviations in Q-Q plots (Supplementary Figure 1, Appendix VI), typical of dietary data, and did not require transformation. However, protein intake, and correspondingly, collagen intake, exhibited slightly greater tail deviations in their respective Q-Q plots (Supplementary Figure 1, Appendix VI). To ensure robustness, protein and collagen intake data were log-transformed, and the analyses were repeated. The results of these transformed analyses were consistent with those using the non-transformed data, suggesting that the observed effects were not influenced by these deviations. Chi-Square tests of independence were performed to examine the association between age group, sex and physical activity levels. Differences in nutritional intake (i.e. energy, protein, carbohydrate, fat, collagen, vitamin C) between age-group and sex were evaluated using one-way analysis of covariance (ANCOVA), with physical activity (PA) level category (low, moderate, high) incorporated as a covariate. Bonferroni adjustment was used for post-hoc comparisons. Partial eta squared ($\eta_p^2$) was reported as an estimate of effect size for ANCOVA main effects and interaction effects. The thresholds of $\eta_p^2$ are defined as small ($\eta_p^2 = 0.01$) medium ($\eta_p^2 = 0.06$) and large ($\eta_p^2 = 0.14$) (Cohen, 2013).

# 3.3 Results

### Collagen sources dataset

The IUNA dataset contained 2552 unique food codes, of which 28.8% (n = 736) were manually identified by the current investigators (CN) to contain collagen. Food codes contained both individual food items and complete recipes. Excluding food codes that

did not contain collagen protein, the collagen composition ($g.100g^{-1}$) of food codes in this database ranged from 0.06 $g.100g^{-1}$ (i.e. soup, chicken, no vegetables) to 5.9 $g.100g^{-1}$ (i.e. bratwurst).

**Habitual collagen intake in Irish adults**

Absolute and relative collagen MDI are displayed in **Figure 1**. The collagen MDI for the entire sample (n = 1338) was 3.2 ± 2.0 g·day⁻¹. There was a main effect of sex on collagen MDI ($F_{1, 1331} = 217.042$, $P < 0.001$, $\eta_p^2 = 0.140$), where intake for males (4.0 ± 2.1 g·day⁻¹; n = 657) was higher than for females (2.4 ± 1.4 g·day⁻¹; mean difference = 1.6 g·day⁻¹; $p < 0.001$). There was also a main effect of age on collagen MDI ($F_{2, 1331} = 3.914$, $p = 0.020$, $\eta_p^2 = 0.006$). Collagen MDI was 3.2 ± 2.0 g·day⁻¹, 3.3 ± 2.0 g·day⁻¹, and 2.9 ± 1.8 g·day⁻¹ for young, middle-aged and older adults, respectively. Post-hoc comparisons revealed a mean difference of 0.4 g·day⁻¹ between middle-aged and older adults (p = .021), but there were no differences between young and middle-aged (p = 1.000), or between young and older adults (p = .053). There was no interaction between sex and age on collagen MDI ($F_{2, 1331} = 1.021$, $p = 0.360$, $\eta_p^2 = 0.002$), and physical activity level had no influence on the statistical model ($F_{1, 1331} = 0.161$, $P = .689$, $\eta_p^2 = .000$).

With regards to relative collagen MDI (collagen intake relative to body mass), the intake for the entire sample (n = 1338) was 0.05 ± 0.03 g·kg⁻¹·day⁻¹. There was a main effect of sex on collagen MDI ($F_{1, 1331} = 70.873$, $p < 0.001$, $\eta_p^2 = 0.052$), with males' intake being higher than for females. Specifically, the males' intake was 0.05 ± 0.03 g·kg⁻¹·day⁻¹, while the females' intake was 0.03 ± 0.02 g·kg⁻¹·day⁻¹ (mean difference = 0.01 g·kg⁻¹·day⁻¹; $p < 0.001$). There was no main effect of age on relative collagen MDI ($F_{2, 1283} = 2.224$, $p = 0.108$, $\eta_p^2 = 0.003$). Intake in young adults was 0.04 ± 0.03

g·kg$^{-1}$·day$^{-1}$, while intake in both middle-aged and older adults was 0.04 ± 0.02 g·kg$^{-1}$·day$^{-1}$. There was no interaction between sex and age on relative collagen MDI ($F_{2, 1283} = 0.97$, $p = 0.379$, $\eta_p^2 = 0.002$). Additionally, physical activity level did not influence the model ($F_{1, 1283} = 0.01$, $p = 0.91$, $\eta_p^2 = 0.000$).

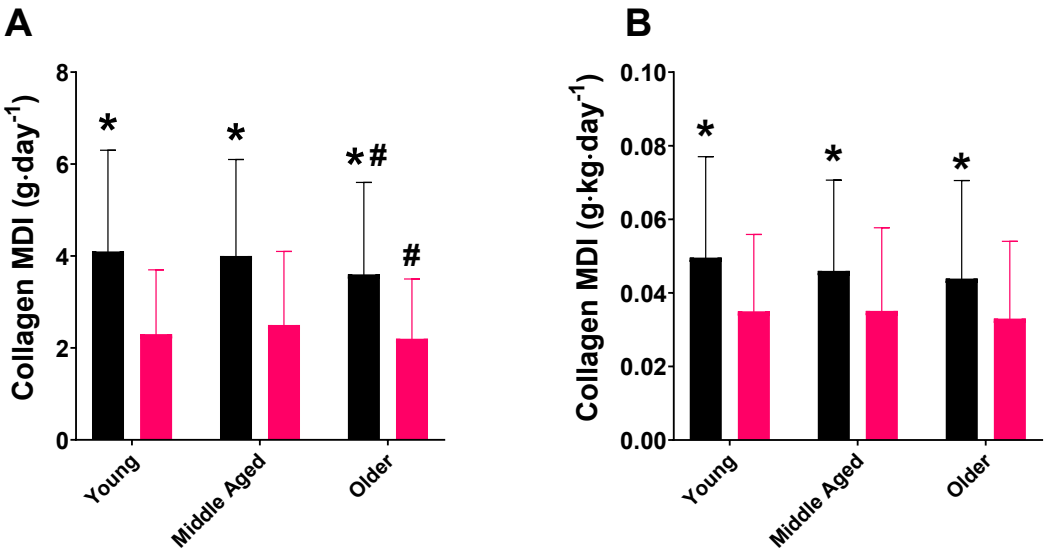


**Figure 3. A.** Absolute and **B.** normalized (to body mass) mean daily intake (MDI) of collagen in young, middle-aged, and older males (black bars) and females (pink bars). * Higher than females ($p < 0.001$), # lower than middle-aged ($p = 0.021$).

Collagen MDI as a percentage of total daily protein intake is displayed in **Figure 2**. There was no main effect of age ($F_{1,1331} = 1.185$, $p = 0.306$, $\eta_p^2 = 0.001$), however, males consumed a greater amount of collagen (4.0 ± 1.9 %) as a proportion of total protein intake compared to females (3.4 ± 1.8 %; $F_{1,1331} = 41.359$, $p < 0.001$, $\eta_p^2 = 0.030$), but there was no age × sex interaction ($F_{1,1331} = 0.470$, $p = 0.625$, $\eta_p^2 = 0.001$). These findings persisted when collagen intake and total protein intake were normalized to body mass (age group: $F_{1,1331} = 0.977$, $p = 0.377$, $\eta_p^2 = 0.002$; sex: $F_{1,1331} = 40.189$, $p < 0.001$), $\eta_p^2 = 0.030$; age × sex interaction: $F_{2,1283} = 0.669$, $p = 0.513$, $\eta_p^2 = 0.001$).

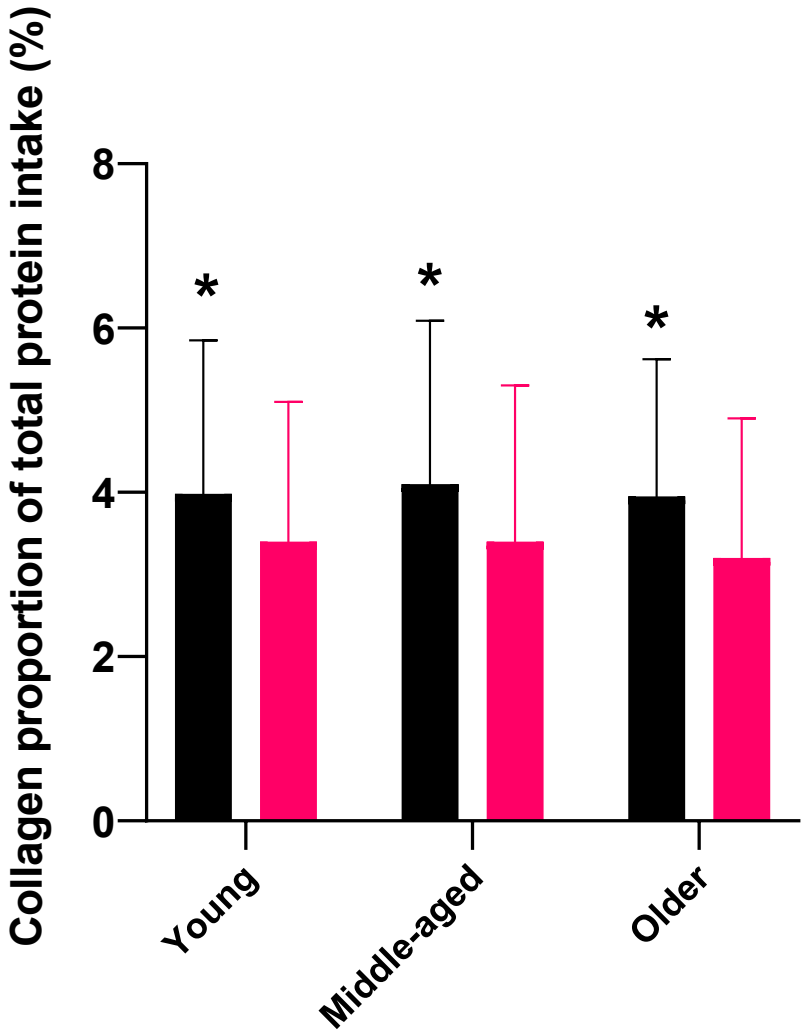


**Figure 2.** Mean daily intake (MDI) of collagen expressed as a percentage of total daily protein intake in young, middle-aged, and older males (black bars) and females (pink bars). * Higher than females ($p < 0.001$).

**Energy and macronutrient intake of Irish adults**

Mean daily energy and macronutrient intake are detailed in **Table 1**. The mean daily energy intake for the entire sample (n = 1,338) was 2,006 ± 639 kcal·day$^{-1}$. There were main effects of age ($F_{2, 1492} = 28.703$, $p < 0.001$, $\eta_p^2 = 0.032$) and sex ($F_{1,1492} = 367.756$, $p < 0.001$, $\eta_p^2 = 0.208$) on daily energy intake, and there was an interaction between age and sex ($F_{2, 1492} = 4.747$, $p = 0.004$, $\eta_p^2 = 0.005$). With regards to absolute intake, males had a higher mean energy intake compared to females across all age groups.

Regarding age, energy intake was lower in older adults compared to both young adults and middle-aged adults. No differences were observed in energy intake between young adults and middle-aged adults for either sex. The interaction was likely a result of the sex difference in middle-aged adults, being smaller than the sex differences in both young and older adults.

When normalized to body mass, the daily energy intake for the entire sample was 26.6 ± 8.7 $kcal \cdot kg^{-1} \cdot day^{-1}$. There were main effects of age group and sex on normalized energy intake ($F_{1,1492} = 31.143$, $p < 0.001$, $\eta_p^2 = 0.022$; $F_{1,1492} = 29.501$, $p < 0.001$, $\eta_p^2 = 0.022$) but no age × sex interaction ($F_{2,\ 1492} = 2.751$, $p = 0.93$, $\eta_p^2 = 0.005$). Normalized energy intake was lower in young compared to middle-aged adults, but there was no difference between middle-aged and older adults. Females had lower normalized energy intake compared with males in all age groups.

Regarding macronutrient intake, there were main effects of age, and sex on protein, carbohydrate and fat intake, respectively ($p < 0.01$). However, there was only an interaction between age and sex on absolute and normalized protein intake ($F_{2,\ 1331} = 9.968$, $p < 0.001$, $\eta_p^2 = 0.015$; $F_{2,\ 1283} = 8.383$, $p < 0.001$, $\eta_p^2 = 0.013$), and not regarding any other macronutrient intake normalized to body mass. Absolute and normalized protein intake was lower in females ($p < 0.001$) but was not different across age groups. Absolute protein intake in males, however, tended to be lower with age (young vs. middle-aged, $p = 0.006$, young vs. old, $p < 0.001$, middle-aged vs. old. $p = 0.064$). Normalized protein intake in males was lower in middle-aged and older compared to young (both groups vs. young, $p < 0.001$), but there was no difference between middle-aged and old ($p = 1.000$). While protein intake was generally consistent across most groups, young males had a notably higher intake compared to their female counterparts and other age groups.

Normalized carbohydrate intake was lower in females, and regardless of sex, intake in young adults was higher than in both middle-aged and older adults, and intake in middle-aged adults was higher than in older adults. There was a main effect of age ($F_{1,1331}$ = 13.482, p < 0.001, $\eta_p^2$ =.020) and sex ($F_{1,1331}$ = 13.482, p <0.001, $\eta_p^2$ =.127) on fat intake, but no sex × age interaction. ($F_{2,\ 1331}$ = 1.122, p = 0.127), which was similar for normalized fat intake (age: p < 0.001, $\eta_p^2$ = .025, sex: p < 0.001, $\eta_p^2$ = .001, sex × age: p = 0.445, $\eta_p^2$ = .001). Physical activity was included as a covariate in the ANCOVA model to account for potential confounding effects. However, this variable did not influence the model for any nutrient apart from fat intake. Physical activity was associated with mean daily fat intake ($F_{1,1331}$ = 4.20, p =.041, $\eta_p^2$ =.003) and normalized fat intake ($F_{1,1283}$ =4.65, p = .031, $\eta_p^2$ =.004), explaining 0.3-0.4% of the variance. This indicates that, while physical activity levels were statistically related to fat intake, its influence on the model was minimal. Importantly, even after adjusting for physical activity, main effects of sex and age on fat intake were observed.

**Table 2.** Habitual energy, protein, carbohydrate, fat, collagen, and vitamin C intake of Irish adults. Values are expressed as mean ± SD.

| Nutrient | | Young adults (n = 596) | Middle-aged adults (n = 575) | Older adults (n = 167) | Effect of Sex | Effect of Age | Sex × Age Interaction |
|---|---|---|---|---|---|---|---|
| **Energy Intake (kcal·day⁻¹)** | All | 2135 ± 692 | 1952 ± 576 | 1808 ± 555 | P < 0.001 | P < 0.001 | P = 0.004 |
| | Male | 2481 ± 656 | 2256 ± 578 | 2086 ± 585 | | | |
| | Female | 1755 ± 506 | 1689 ± 425 | 1564 ± 391 | | | |
| **Energy Intake (kcal·kg⁻¹·day⁻¹)** | All | 28.8 ± 9.3 | 25.2 ± 7.5 | 26.7 ± 8.7 | P < 0.001 | P < 0.001 | P = 0.93 |
| | Male | 30.5 ± 9.5 | 26.1 ± 7.6 | 25.5 ± 8.9 | | | |
| | Female | 26.8 ± 8.7 | 24.2 ± 7.6 | 23.8 ± 7.3 | | | |
| **PRO Intake (g·day⁻¹)** | All | 87 ± 31 | 84 ± 25 | 79 ± 24 | P = 0.005 | P < 0.001 | P < 0.001 |
| | Male | 104 ± 31 | 97 ± 24 | 91 ± 24 | | | |
| | Female | 69 ± 20 | 73 ± 20 | 69 ± 18 | | | |
| **PRO Intake (g·kg⁻¹·day⁻¹)** | All | 1.2 ± 0.4 | 1.1 ± 0.3 | 1.1 ± 0.4 | P < 0.001 | P = 0.002 | P < 0.001 |
| | Male | 1.3 ± 0.4 | 1.1 ± 0.3 | 1.1 ± 0.4 | | | |
| | Female | 1.0 ± 0.3 | 1.1 ± 0.3 | 1.0 ± 0.4 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **CHO Intake (g·day$^{-1}$)** | All | 243 ± 82 | 225 ± 76 | 214 ± 69 | P < 0.001 | P < 0.001 | P = 0.053 |
| | Male | 278 ± 84 | 257 ± 83 | 240 ± 74 | | | |
| | Female | 205 ± 60 | 197 ± 57 | 191 ± 54 | | | |
| **CHO Intake (g·kg$^{-1}$·day$^{-1}$)** | All | 3.3 ± 1.1 | 2.9 ± 1.0 | 2.9 ± 1.0 | P = 0.001 | P < 0.001 | P = 0.277 |
| | Male | 3.4 ± 1.2 | 3.0 ± 1.1 | 3.0 ± 1.0 | | | |
| | Female | 3.1 ± 1.0 | 2.9 ± 1.0 | 2.8 ± 1.0 | | | |
| **Fat Intake (g·day$^{-1}$)** | All | 81 ± 30 | 75 ± 27 | 70 ± 29 | P = 0.001 | P < 0.001 | P = 0.326 |
| | Male | 92 ± 30 | 86 ± 29 | 80 ± 33 | | | |
| | Female | 68 ± 24 | 66 ± 21 | 61 ± 20 | | | |
| **Fat Intake (g·kg$^{-1}$·day$^{-1}$)** | All | 1.3 ± 0.5 | 1.2 ± 0.5 | 1.1 ± 0.5 | P < 0.001 | P < 0.001 | P = 0.445 |
| | Male | 1.4 ± 0.5 | 1.3 ± 0.5 | 1.2 ± 0.6 | | | |
| | Female | 1.2 ± 0.5 | 1.1 ± 0.4 | 1.0 ± 0.5 | | | |
| **Vitamin C Intake (mg·day$^{-1}$)** | All | 134 ± 279 | 119 ± 175 | 132 ± 215 | P = 0.020 | P = 0.516 | P = 0.575 |
| | Male | 127 ± 167 | 104 ±130 | 104 ± 134 | | | |
| | Female | 141 ± 363 | 131 ± 205 | 141 ± 291 | | | |

PRO, protein; CHO, carbohydrate; young adults were 18-39 years-old; middle-aged adults were 40–64 years-old; older adults were >65 years-old.

**Habitual vitamin C intake of Irish adults**

Vitamin C MDI is presented in **Table 2**. There was no main effect of age on vitamin C MDI ($F_{2, 1331} = 0.661$, p = 0.516), and no age × sex interaction ($F_{2, 1331} = 0.554$, p = 0.575). However, there was a main effect of sex ($F_{2, 1331} = 5.448$, p = 0.020, $\eta_p^2 = 0.004$), where females' intake (141 ± 291 mg·day$^{-1}$) was higher than for males (115 ± 151 mg·day$^{-1}$) (p < 0.001). Physical activity had no influence on the ANCOVA model ($F_{1, 1331} = 0.239$, p = 0.625).

**Habitual physical activity levels of Irish adults**

Although the NANS included 1,500 respondents in its dataset, 162 cases were excluded from this study due to incomplete physical activity questionnaires, leaving a final 1,338 valid cases which were used for analysis. From the final analysed sample, 1,135 cases were in the 'low' PA category, with 534 (47.0%) males and 601 (53.0%) females. The age distribution of this PA sub-group was as follows: 475 (41.9%) young (231 males, 244 females), 495 (43.6%) middle-aged (226 males, 269 females), and 165 (14.5%) older (77 males, 88 females). There was no significant association between age group and sex in the 'low' PA category ($\chi^2$ (2, n = 1,135) = 0.958, p = 0.619).

Similarly, there was no significant association between age group and sex in the 'moderate' PA category ($\chi^2$ (2, n = 184) = 2.614, p = 0.271). Of the 184 cases in the 'moderate' PA category, 109 (59.2%) were males and 75 (40.8%) were females. The age distribution was 108 (58.7%) young (69 males, 39 females), 75 (40.8%) middle-aged (40 males, 35 females), and 1 (0.5%) older (0 males, 1 female).

In contrast, the analysis for the 'high' PA category revealed a significant association between age group and sex ($\chi^2$ (2, n = 19) = 8.051, p = 0.018). Specifically, there was

a higher proportion of young adults engaged in ‘high’ PA compared to middle-aged and older adults. Additionally, the sex distribution within the ‘high’ PA category differed notably, with males being more represented among the younger age group compared to females. For the 19 cases in this category, there were 14 (73.7%) males and 5 (26.3%) females. The age distribution was 12 (63.1%) young (12 males, 1 female), 5 (26.3%) middle-aged (2 males, 3 females), and 1 (5.3%) older (0 males, 1 female). Finally, the aggregate data across all physical activity categories revealed no overall association between age group and sex ($\chi^2$ (2, n = 1,338) = 4.534, p = 0.104).

## 3.4 Discussion

The main objective of this study was to provide the first estimate of collagen intake in a European adult population, based on data from the National Adult Nutrition Survey (NANS). The main findings were that the collagen mean daily intake (MDI) for the entire study population was ~ 3 g per day, which represented just ~ 4 % of all protein consumed daily. This intake is considerably lower than the doses necessary to enhance collagen synthesis in intervention studies. Specifically, in young men, 15 g but not 5 g of gelatin increased whole-body collagen synthesis following skipping exercise (Shaw *et al.*, 2017), while 30 g, but not 15 g of collagen hydrolysate was required to enhance collagen synthesis following resistance exercise (Lee *et al.*, 2023c). This suggests that the levels of collagen habitually consumed in the Irish diet are likely insufficient to elicit a meaningful collagen synthesis response. Interestingly, in our study, men had greater absolute intakes of collagen than women regardless of age, and this sex difference remained when intakes were adjusted for body mass. Furthermore, older adults consumed less collagen than middle-aged adults in absolute terms (with no difference between middle-aged and young adults) but this age difference

disappeared when collagen intake was normalised to body mass. Notably, habitual collagen MDI was not influenced by habitual physical activity levels, i.e., more physically active individuals did not ingest more collagen.

The collagen MDI for the total population was remarkably low, thus the availability of exogenous glycine, proline, and hydroxyproline (the highly abundant amino acids in collagen known to stimulate collagen synthesis (Szoka *et al.*, 2017; de Paz-Lugo *et al.*, 2018)) is also limited. Glycine is not an essential amino acid, as it can be synthesized from other amino acids (predominantly serine, but threonine, choline and glyoxylate may make minor contributions) (Meléndez-Hevia *et al.*, 2009; Wang *et al.*, 2013). However, habitual protein/amino acid intake plays a role in supplying glycine, proline, and hydroxyproline to support connective tissue turnover in the skin, heart, blood vessels, and musculoskeletal tissue, although it has been shown that dietary glycine intake between 1.5 and 3 g·day$^{-1}$ falls short of the amount required for collagen synthesis in metabolism (Meléndez-Hevia *et al.*, 2009). Although glycine may be available from some food sources, such as soy or legumes, the amount of glycine available from the ~ 3 g MDI collagen in the Irish diet, is likely to be as low as 1 g·day$^{-1}$ since glycine comprises 1/3 of collagen (Ramshaw *et al.*, 1998).

Moreover, collagen is the only dietary source of hydroxyproline. It has been demonstrated in human dermal fibroblasts, that hydroxyproline stimulates collagen synthesis in two ways: 1. by increasing growth factor beta 1 (TGF β1) levels; and 2. by directly stimulating the protein kinase B (AKT) and mammalian target of rapamycin (mTOR) signaling pathways (Surazynski *et al.*, 2010). Given this crucial role in supporting connective tissue turnover, the unique presence of hydroxyproline in collagen further emphasizes the importance of including collagen in the diet.

Despite this importance, the only previously reported habitual intakes of collagen were the 3 to 23 g·day$^{-1}$ in 'the standard American diet' reported by Paul *et al.* (2019). There are several reasons for the discrepancies between our data and those of Paul *et al.* (2019), where our data are much closer to the lower end of collagen MDI estimates in the study by Paul *et al.* (2019). There are crucial differences in methodology, where Paul *et al.* (2019) estimated collagen MDI by averaging collagen content (% dry weight) across multiple food groups, and expressed this as a percentage of mean male and female intake at population level, as calculated in the National Health and Nutrition Examination Survey (NHANES). In contrast, our study applied specific collagen content values to 736 food items on an individual basis and integrated these into the food diaries of each participant in NANS. For example, Paul *et al.* (2019) grouped beef, pork, veal, lamb and game, and assigned these foods a collagen content of 5.15 % of product dry weight, however the collagen content of pork cuts alone can vary from ~1 to ~22 % (FSAI, 2018). Moreover, any of these foods could be included in a food mixture, which is not accounted for in these analyses by Paul *et al.* (2019). Finally, the higher end of collagen MDI range reported in the NHANES may be attributed to differences in regional food regulation, as European Union Regulation (EU) No. 1169/2011 sets out maximum connective tissue content (measured as collagen content) for ingredients designated by the term 'meat' at 25 % (FSAI, 2018). In stark contrast, Paul *et al.* (2019) reports the collagen content of frankfurters, sausages and luncheon meat in to be ~ 55% in the United States of America (USA), highlighting the lack of applicability of these findings across different jurisdictions, and the likelihood that collagen intake is lower in Europe compared to the USA.

We found that dietary collagen intake was lower in Irish female adults, both in absolute terms and relative to both body mass and total protein intake. Using the same dataset

as used in this study, Hone et al. (Hone *et al.*, 2020) recently reported that animal based foods contributed to a larger proportion of total energy and were the dominant source of protein intake in the Irish adult diet. However, women obtained a higher proportion of protein from plant sources compared to men (Hone *et al.*, 2020), and since collagen is exclusively found in animal products, this may explain the lower relative collagen intake in women, despite similar relative protein intake. Although the mean difference in total collagen intake between men and women appears modest (1.6 $g \cdot day^{-1}$), the large effect size ($\eta_p^2 = 0.140$) suggests that future research should explore sex-specific recommendations for increasing collagen intake, with the goal of improving connective tissue health, especially in women.

The lower daily protein intake observed in older adults aligns with findings in other Western European jurisdictions (Sette *et al.*, 2011; Ruiz *et al.*, 2015; Tieland *et al.*, 2015). It is striking, however, that there was an age × sex interaction regarding protein, and not collagen, intake relative to body mass. There are known effects of age and sex on collagen turnover in healthy humans, especially in type I collagen, the main extracellular matrix component of bone, tendon, and ligament (Glover *et al.*, 2008; Morovat *et al.*, 2013; Kehlet *et al.*, 2018a). Biomarkers of type I collagen synthesis decline from young adulthood until middle age before levelling off in both men and women (Glover *et al.*, 2008; Morovat *et al.*, 2013). Moreover, young and middle-aged women display lower levels of collagen synthesis than men, with lowest levels reported in middle-aged, pre-menopausal women, and an increase in the post-menopausal years (Glover *et al.*, 2008). These observational data suggest a role for hormonal status on collagen turnover. Consequently, middle-aged and older Irish women in particular, who also have the lowest intake of collagen according to our data, may have different dietary collagen requirements to young adults. Additionally,

study designs that seek to measure the effects of dietary collagen should avoid grouping male and female participants, as differences in collagen turnover and hormonal status, even in age-matched participants, are likely to lead to erroneous conclusions.

We estimated habitual vitamin C intake in the Irish adult population, as it is essential for the hydroxylation of proline and lysine, a crucial step in the synthesis of collagen (Canty & Kadler, 2005). Although vitamin C intake was ~23 % greater in women than men, our sex-specific values were similar to the recommended dietary allowance (RDA) of 95 and 110 $mg \cdot day^{-1}$ for women and men, respectively (EFSA Panel on Dietetic Products & Allergies, 2013). However, it remains unclear whether the timing of vitamin C ingestion (for example co-ingestion with collagen rich food or supplements) is essential to optimize collagen synthesis in response to feeding. Despite adequate daily intake, the inability of humans to store vitamin C (Li & Schellhorn, 2007), suggests that any discontinuity between intake of vitamin C and collagen could potentially limit the level of endogenous collagen production.

**Physical activity levels**

We used habitual physical activity as a covariate when analysing differences in collagen intake across age groups due to the recent interest in the interaction between collagen supplementation and various types of exercise (Khatri *et al.*, 2021; Bischof *et al.*, 2024). Although not the primary outcome of our study, it is concerning that 85 % of all participants were in the low activity category, with low levels of moderate physical activity in middle-age, and indeed that only one older adult could be classified as highly active. This aligns with international data indicating lower physical activity levels among older populations (Posadzki *et al.*, 2020; Kettle *et al.*, 2022). A recent

meta-analysis suggested that chronic exercise with collagen ingestion can improve fat-free mass, tendon and muscle morphology, maximal strength and recovery from damaging exercise bouts (Bischof *et al.*, 2024). Since the current study is a secondary analysis of questionnaire based physical activity, we were not able determine whether moderate or highly active participants were engaging in resistance exercise, endurance exercise, or other activities. The observation that habitual physical activity was lower in middle-aged adults compared to young, and none of the older adults were highly active, supports the notion that the combined effects of increased collagen ingestion and exercise may have the greatest benefit in terms of stimulating collagen production for maintaining or improving connective tissue health in middle-aged and older populations.

**Strengths and Limitations**

A key strength of this study is the application of specific collagen content values to individual food items, which allowed for a more precise estimation of collagen intake compared to a previous population-level assessment that relied on broad food group averages. Additionally, these data are derived from a demographically representative sample, allowing generalizability to the Irish adult population. Our analysis is from the Irish NANS, conducted between 2008 and 2010. Dietary habits and supplement use may have changed since then, given the growth in global sales of collagen supplements and increased research in active and athletic groups. It could be suggested that collagen intake may be increasing or will increase in future, surpassing the most recent data available for the Irish adult population. The study achieved a response rate of 60 %, which is relatively high but still leaves room for potential non-response bias. The sample was demographically representative, but specific sub-groups (e.g., highly

active middle-aged and older adults) were small, limiting the generalizability of findings to these populations.

**Conclusion**

Habitual intake of collagen protein was remarkably low in the diet of Irish adults and may fall short of that required for optimal collagen turnover to maintain healthy connective tissues. Since collagen ingestion with exercise may improve musculoskeletal health and function, increasing collagen ingestion may be an effective strategy for maintaining connective tissue health. Achieving effective doses between 5 and 30 g through diet alone may be challenging, therefore, supplementation may be warranted. This may be especially important for women and older adults, who typically consume less collagen than men and younger adults, according to our data.

# Chapter Four

# Hydrolysed collagen supplementation prior to resistance exercise augments collagen synthesis in a dose-response manner in resistance-trained, middle-aged men

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## Prelude

Chapter Three focused on the habitual dietary intake of collagen in Irish adults. The main finding was that collagen intake comprised only 4% of total protein intake (~4 $g \cdot day^{-1}$ in men), which tended to decrease with age. It is known that 30 g, but not 15 g HC augments the collagen synthesis response to RE in young, resistance-trained men, but this has not been examined in older age groups. This chapter will therefore investigate the effect of 0 g vs. 15 g vs. 30 g HC ingested prior to high-intensity leg press resistance exercise on biomarkers of collagen turnover in resistance-trained, middle-aged men. This study was performed in two parts, separated by national lockdown laboratory closure (due to the COVID-19 pandemic). The initial data collection took place between November 2019 and March 2020, and was resumed and completed between July 2022 and October 2022.

# Abstract

Resistance exercise (RE) increases collagen synthesis in young and older men, while hydrolysed collagen (HC) ingestion improves this response to RE in a dose-response manner in young men. However, the collagen synthesis response to RE with and without HC in *middle-aged* men is unknown. Eight resistance-trained men (age: 49 ± 8 years; height: 1.78 ± 0.02m; mass: 90±4 kg) took part in this double-blind, crossover design study, and undertook 4×10 repetitions of lower-limb RE at maximum load, after consuming 0g, 15g, or 30g vitamin C-enriched HC. We analysed venous blood samples for N-terminal propeptide of type 1 pro-collagen (PINP), β-isomerized C-terminal telopeptide of type 1 collagen (β-CTx) and 18 collagen amino acids throughout all three interventions. The serum PINP concentration × time area-under-the-curve (AUC) was higher following 30g (169 ± 28 μg/mL×h) than 15g (134 ± 23 μg/mL×h, $P < 0.05$) HC ingestion, and both 15g and 30g were higher than 0g HC (96 ± 23 μg/mL×h, $P < 0.05$). RE with 0g HC showed no change in serum PINP concentration. The AUCs for glycine, proline, hydroxyproline, alanine, arginine, lysine, serine, leucine, valine and isoleucine were greater with 30g than 15g and 0g HC ingestion ($P < 0.05$), and greater with 15g than 0g HC ingestion ($P < 0.05$). Plasma β-CTx concentration decreased after RE independently of HC dose. Our study suggests connective tissue anabolic resistance to RE in middle-aged men but ingesting 15g HC rescues the collagen synthesis response, and 30g augments that response further. This dose-response is associated with the increased bioavailability of collagen amino acids in the blood, which stimulate collagen synthesis.

# 4.1 Introduction

Injury is a major concern for athletes of all ages, with injury rates to collagenous tissues such as muscle, ligament and tendon being similar between young, middle-aged and older male athletes (Ganse *et al.*, 2014). Chronic resistance exercise (RE), however, is a safe and effective method to reduce tendon injury risk (Lauersen *et al.*, 2018). For example, chronic high intensity RE causes the tendon of healthy adults to adapt by increasing its mechanical (stiffness) and morphological (size) properties (Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009; Bohm *et al.*, 2015; Wiesinger *et al.*, 2015b), which should result in reduced tissue stress during loading (LaCroix *et al.*, 2013). Furthermore, increasing tendon stiffness can improve the rate of torque development (RTD) (Bojsen-Møller *et al.*, 2005), which should enhance athletic performance (Lichtwark & Wilson, 2005; Stafilidis & Arampatzis, 2007) by enabling force to be transmitted from the muscle to the bone more efficiently (Wang *et al.*, 2012).

The RE-induced change in tendon stiffness can be explained by an increase in tendon cross-sectional area (Heinemeier & Kjaer, 2011), an increase in collagen fibril density (Couppé *et al.*, 2021) and an increase in collagen fibril cross-linking (Couppe *et al.*, 2009; Stammers *et al.*, 2020). For the first two of these factors to occur, an increase in collagen synthesis is likely required for collagen content to increase (Kjaer *et al.*, 2006; Chiquet *et al.*, 2009; Mousavizadeh *et al.*, 2020; Crossland *et al.*, 2023). As human tendon dry weight comprises 60-85% type I collagen (Hanson & Bentley, 1983; Kjaer, 2004), measuring serum concentration of procollagen type I N-terminal propeptide [PINP, which is cleaved off during the maturation of procollagen to collagen] can indicate whether tendon collagen synthesis has increased following RE. Indeed,

previous studies have found that serum PINP concentration increased following a single bout of RE (Huang *et al.*, 2022a; Lee *et al.*, 2023b) and after 12 weeks' RE (Mosti *et al.*, 2014).

Given the importance of a positive net collagen turnover for RE-induced connective tissue adaptations to occur (Kjaer *et al.*, 2006; Chiquet *et al.*, 2009; Mousavizadeh *et al.*, 2020; Crossland *et al.*, 2023), exogenous collagen ingestion may enhance the collagen synthesis response to an acute bout of RE. Indeed, Lee et al. (Lee *et al.*, 2023b) recently showed that HC ingestion prior to high-intensity RE augmented the serum PINP concentration × time area under the curve (AUC) in a dose-response manner in resistance-trained, *young* men, i.e. 30 g > 15 and 0 g. This response was likely related to the higher postprandial serum concentrations of key amino acids associated with collagen composition and synthesis (e.g. glycine, proline, and hydroxyproline) in the 30 g intervention compared to the 15 g and 0 g interventions (Lee *et al.*, 2023b). However, this is the only study to date to have investigated the dose-response relationship of collagen ingestion prior to RE on collagen synthesis, and this relationship may be different in other populations. For example, it is not yet known how much collagen is necessary to maximize collagen synthesis following RE in middle-aged men.

Research typically contrasts extreme age groups to accentuate ageing effects on RE and nutrition, leaving the middle-aged population (40 - 64 years) often overlooked. For instance, compared to young adults (aged 18 – 39 years), tendons of older adults (aged > 65 years) show diminished extracellular matrix gene expression in response to acute RE (Crossland *et al.*, 2023). While older tendon improves with chronic RE, the rate of adaptation is slower than younger tendon (Quinlan *et al.*, 2021). It is crucial, however, to examine responses in middle-aged individuals, to provide meaningful

exercise and nutritional recommendations for this population. Ageing is known to blunt the anabolic response to RE and protein ingestion, doubling the protein intake required to optimize muscle protein synthesis after RE in older adults compared to young (Moore *et al.*, 2009; Yang *et al.*, 2012). Given that collagen-rich tissues such as skin exhibit reduced collagen synthesis with age (Varani *et al.*, 2006), it is possible that ageing also alters the collagen synthesis response to exogenous collagen ingested before RE, although this remains to be explored.

The aim of this study was to investigate the effect of lower-limb RE with vitamin C-enriched HC supplementation on serum PINP concentration in resistance-trained, middle-aged men. The objective was to determine the optimal dose of HC required to stimulate maximal whole body collagen synthesis following RE in this population. We hypothesized that collagen synthesis would increase in response to RE, and that this effect would be augmented in a dose-dependent manner by supplementation with vitamin C-enriched HC.

# 4.2 Methods

***Participants***

Participants were recruited from a population of resistance-trained, middle-aged men (i.e. posters advertising the study were placed in gymnasiums of the local area, and local sports clubs were emailed with the study information, which was disseminated to members). Recruitment began in November 2019 and data collection was completed in September 2021. To be eligible to participate, volunteers had to be male, have at least 12 months' resistance training experience (including lower limb resistance exercise [RE] performed at least once a week) and to be free from

musculoskeletal injury. Volunteers were excluded if they had a history of patellar tendon pathology, were vegan (due to the bovine source of HC), consumed nutritional supplements or medication purported to have beneficial effects on muscle-tendon properties (e.g. antioxidants, protein, etc.), had sustained a lower limb injury in the previous six months, smoked or were <40 or >65 years-old. The required sample size was estimated before conducting the study with G*Power software (version 3.1.9.6, Heinrich-Heine-Universität Düsseldorf). The *a priori* estimation was performed using a large effect size ($\eta_p^2 = 0.22$), on the basis of the results from Shaw et al. (2017), which demonstrated a two-fold increase in the serum PINP concentration × time AUC after exercise with 15 g compared to 5 g gelatine ingestion. A minimum of eight participants was deemed necessary to detect an effect of HC dose [one-way repeated measures analysis of variance (ANOVA); α: 0.05; power: 0.80]. To account for participants withdrawing from the study, 13 resistance-trained, middle-aged men were recruited. However, three participants withdrew prior to commencement of the first intervention, one based on recommendation from his general practitioner, and two due to injuries sustained independent of the study. Two more participants completed a portion of the study in March 2020, however, due to immediate laboratory closure during the COVID-19 national lockdown restrictions in Ireland, they could not complete all interventions and were subsequently excluded from the analysis. Therefore, eight men (mean ± SD: age, 49 ± 8 years; height, 178 ± 2 cm; body mass, 90 ± 4 kg; 10 repetition maximum (10-RM) leg press, 330 ± 88 kg) were included in the final analyses after providing written informed consent prior to study commencement (Figure 1). The study was registered at https://clinicaltrials.gov/ (identifier: NCT06236659), was approved by Liverpool John Moores University

Research Ethics Committee (approval number: 19SPS049) and complied with the Declaration of Helsinki.

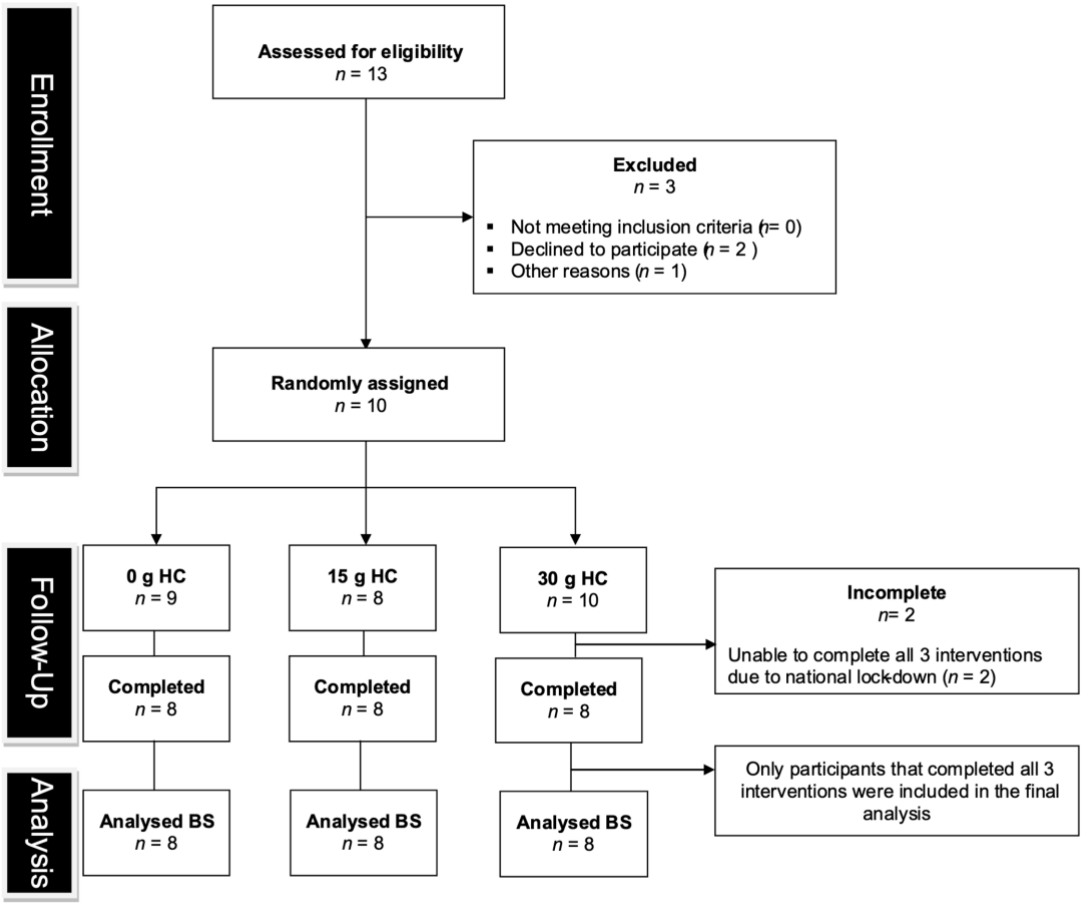


**Figure 1**. CONSORT diagram. *HC,* hydrolysed collagen intervention; *BS*, blood sample.

### *Experimental Design*

The study was a double-blind, repeated measures crossover design. Participants reported to the laboratory on four separate occasions separated by 72 hours. The purpose of the initial visit was to establish each participant's 10-RM on a leg press machine (Samson, New Mexico, USA), and to familiarize them with the exercise protocol. Secondly, body mass and stature were measured using calibrated weighing

scales (model 769, SECA, Birmingham, UK) and a wall mounted digital stadiometer (model 264, SECA, Birmingham, UK), respectively. The order of measurements during familiarization was the same as the order they appear below. During the subsequent three visits, participants reported to the laboratory at 07:00 after a 10 h overnight fast and ingested either 0, 15, or 30 g vitamin C-enriched hydrolysed collagen (HC) supplement in a randomized order, prior to four sets of 10-RM on the leg press machine. This was followed by 6 h fasted, passive (seated) rest. Venous blood samples were collected at regular intervals for the duration of each intervention (Figure 2), and serum/plasma samples were analysed for the concentration of procollagen type I N-terminal propeptide (PINP, a marker of collagen synthesis), 18 collagen amino acids, and β-isomerized C-terminal telopeptide (β-CTx, a marker of collagen breakdown).

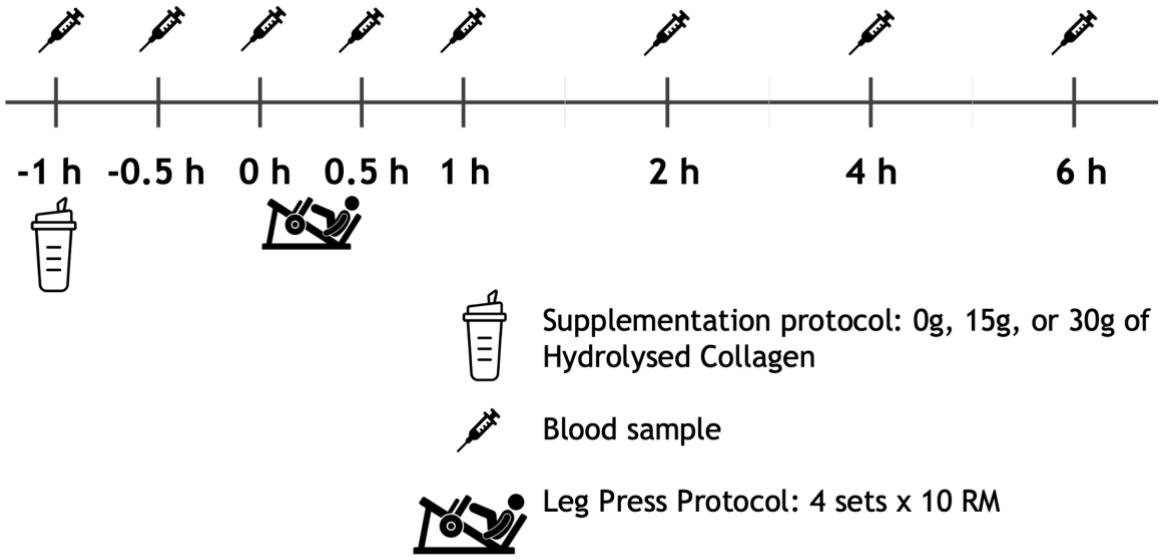


**Figure 2**. Schematic timeline of data collection.

### *Leg Press 10 Repetition Maximum (10-RM) Protocol*

Following a brief dynamic warm-up, comprising 5 min submaximal cycling and 5 min full body dynamic stretching, participants performed 2 – 4 sets' leg press RE (Samson, New Mexico, USA) with no additional load, in order to determine their individual foot placement and range of motion on the machine. The 10-RM was determined during four stages: participants first performed 1 – 2 sets of 5 – 10 repetitions at an estimated 50% - 70% of their perceived 10-RM. Following this, the load was increased by 5 – 20 kg on each subsequent set for a total of 4 – 6 attempts, separated by 3 min rest until the participant could only perform 10 repetitions or fewer. The final load, for which the participant successfully completed 10 repetitions, was deemed to be their 10-RM.

### *Dietary Control Measures*

Habitual dietary behaviour of all participants was measured to ensure participants maintained their normal diet throughout the three nutritional interventions, and to ensure the same meal was consumed on the night prior to each intervention. To provide an estimate of habitual macronutrient and energy intake, participants were required to complete a dietary log of all food and beverages consumed on two weekdays and one weekend day (e.g. Thursday, Friday and Saturday) during the week prior to the first intervention (Table 1). Instructions and reference guides to assist in estimating portion sizes were provided. Estimates of nutrient and energy intake were assessed using nutritional software (Nutritics v5.80, Nutritics LTD, Dublin, Ireland). Before each intervention, participants were requested to abstain from caffeine and alcohol in the preceding 24 h and to finish consuming their final meal no later than 21:00 in the evening. In between interventions, participants were asked to continue monitoring their food intake, and to inform the researchers of any deviation from their food

diaries. Electronic reminders to maintain the same intakes were sent to each participant on the days between interventions, where they were also asked to confirm their compliance. A final electronic reminder was sent to each participant at 20:00 the night before each intervention, stating that they should consume the same final meal and begin fasting from 21:00. Participants were instructed to fast overnight (water could be consumed ad libitum) and report to the laboratory at 07:00 the following morning.

**Table 1.** Mean daily energy, macronutrient, and vitamin C intake for all participants (n=8).

| | |
|---|---|
| Energy (Kcal/day) | 2378 ± 757 |
| Carbohydrate (g/day) | 242 ± 48 |
| Carbohydrate (g/kg/day) | 2.7 ± 0.5 |
| Protein (g/day) | 153 ± 95 |
| Protein (g/kg/day) | 1.7 ± 1.1 |
| Fat (g/day) | 81 ± 40 |
| Fat (g/kg/day) | 0.9 ± 0.4 |
| Vitamin C (mg/day) | 127 ± 105 |

Values represent mean ± SD.

***Supplementation with vitamin C-enriched collagen hydrolysate***

The supplement beverages were made by a laboratory technician (independent to this study), using opaque bottles to ensure that both the researchers and participants remained blinded. The supplement in each of the three interventions contained either 0, 15, or 30 g unflavored hydrolyzed collagen (HC) powder (Collagen Protein, MyProtein, Manchester, UK), together with 50 mg vitamin C powder (Holland and Barrett, Dublin, Ireland) and 400 mL water. To ensure the supplement in each of the

three interventions remained isocaloric, 30.5 g and 15.3 g maltodextrin (MyProtein, Manchester, UK) were added to the 0 g and 15 g doses of HC, respectively. To match each supplement for taste, 3 g non-caloric sweetener (Pure Via 100% Xylitol, Mersiant UK LTD., Buckinghamshire, UK) was added to the 0 g dose, and 4 g was added to both the 15 g and 30 g HC doses.

***Resistance exercise intervention and blood sampling***

Upon arrival at the laboratory, participants rested for 15 min before an indwelling cannula (BD Nexiva™, 22G, BD Medical, Berkshire, UK) was inserted into an antecubital vein to allow for repeated blood sampling. A baseline blood sample was collected (-1 h), into 5 mL serum and plasma separating tubes (BD Medical, Berkshire, UK), and the participant was instructed to consume the entire contents of the supplement within 5 min of that blood sample being taken. After ingestion, participants rested for 1 h while additional blood samples were drawn at two more time points (-0.5 h and 0 h). Participants then completed a standardized warm up, comprising dynamic stretching, and two ascending warm-up sets at 50-80% 10-RM on the leg press machine (Samson, New Mexico, USA). The RE intervention comprised 4 sets of 10 repetitions at 90-100% 10-RM, with each set separated by 2 min rest. Having completed the RE, participants were required to remain in a rested state in the laboratory for the subsequent 6 h, while refraining from eating and drinking, apart from ingesting water *ad libitum*. Blood samples were drawn at eight time points over the 7-h period (-1 h, -0.5 h, 0 h, +0.5 h, +1 h, +2 h, +4 h, +6 h, Figure 2). The cannula was flushed with 5 mL saline solution every 30 min to prevent clotting. Whole blood was collected in 5 mL serum separating tubes (BD Medical, Berkshire, UK) at all time points, and allowed to clot for at least 30 min. At time points -1 h, +0.5 h, + 2 h, and +6 h, additional whole blood samples were collected in 5 mL plasma

separating tubes (BD Medical, Berkshire, UK). Whole blood samples were centrifuged at 2000 revolutions per minute (RPM) for 10 min, before being aliquoted into 1.5 mL Eppendorf tubes and stored at −70°C until subsequent analysis.

***Blood analyses***

The methods for measuring markers of collagen synthesis and breakdown, and concentrations of circulating collagen amino acids, have been described in detail previously (Lee *et al.*, 2023b), and are therefore described here in brief. PINP analyses were performed at South East Technological University, while β-CTX and amino acid profile analyses were performed at the Bioanalytical Facility, University of East Anglia.

***PINP and β-CTx***

Serum samples at rest prior to HC ingestion, 0.5 h-post RE, 1-h post RE, 2 h-post RE, 4 h-post RE and 6 h-post RE were used to measure serum PINP concentrations using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Cloud-Clone Corp, Wuhan, China). The intra-assay coefficient of variation (CV) was <10% and the inter-assay CV was <12%, with a detection range of 2.47-200 $\mu g \cdot L^{-1}$, and sensitivity of <0.91 $\mu g \cdot L^{-1}$. The ELISA absorbance readings were performed at 450 nm, using a Versamax microplate reader (Molecular Devices Corporation, Sunnyvale, California USA). The concentration × time total area under the curve (AUC) for PINP and amino acids (see below) were calculated using Prism software (version 9.4.1, GraphPad Inc., San Diego, San Diego, California USA). EDTA plasma concentrations of β-CTx were measured using electrochemiluminescence immunoassay on a Cobas e601 analyser (Roche

Diagnostics, Germany). The inter-assay coefficient of variation (CV) for β-CTx was ≤ 3% between 0.2 and 1.5 µg/L with the sensitivity of 0.01 µg/L.

***Amino acid profile by LC-MS/MS analysis***

Eighteen amino acids associated with collagen composition (lycine, proline, hydroxyproline, glutamic acid, alanine, arginine, aspartic acid, lysine, serine, leucine, valine, phenylalanine, threonine, isoleucine, histidine, tyrosine, methionine, and glutamine) were measured simultaneously using anionic ion-pair reverse phase liquid chromatography tandem mass spectrometry system following derivatization of the amino acid with *n*-butanol hydrogen chloride. The assay range was 0 – 2000 nmol/L for alanine, glutamine, glutamic acid, glycine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine, whereas for arginine, histidine, hydroxyproline, isoleucine, methionine and ornithine the assay range was 0 – 500 nmol/L. Inter-assay precision coefficient of variation (CV) for all amino acids studied were between 3.3% to 10.3%.

***Statistical Analysis***

All statistical analyses were conducted while researchers were still blinded to participant information and supplement dose. Data were analysed using GraphPad Prism, version 9 (San Diego, California, USA) and are presented as mean ± SD unless otherwise stated. Serum amino acid concentrations were analysed using two-factor (time × supplement dose) repeated measures ANOVAs. The same analyses were performed on total PINP concentrations over time, and Tukey's post hoc test was computed for post hoc comparisons. The concentration × time total area under the curve (AUC) was computed for serum PINP and for each amino acid, and compared

between interventions using a one-way repeated measures ANOVA with Tukey's post-hoc analyses performed for multiple pairwise comparisons.

## 4.3 Results

***Effects of collagen supplementation combined with resistance exercise on collagen synthesis in men***

Serum PINP concentration at baseline did not differ between interventions (0 g: 11.7 ± 9.3 µg/L; 15 g: 13.3 ± 7.3 µg/L; 30 g: 11.3 ± 8.7 µg/L; $F_{2,9} = 0.126$, $P = 0.882$). There was a main effect of time ($F_{7,49} = 6.51$, $P < 0.001$) and dose ($F_{2,14} = 18.3$, $P < 0.001$). There was also a dose × time interaction effect ($F_{14,98} = 3.54$, $P < 0.001$), as there were no changes across time in the 0 g HC intervention, but there were changes in serum PINP concentration during the 15 g and 30 g HC interventions. Serum PINP concentration was higher at all time points after RE during the 30 g HC intervention compared with the 0 g intervention (Figure 3A, $P < 0.05$), whereas 15 g was higher than 0 g at + 0.5 h and 1 h only ($P < 0.05$). In both the 15 g and 30 g HC interventions, PINP concentrations peaked at 2 h after RE. Peak PINP values were 70 % and 146 % higher than 0 g at 2 h post-exercise in the 15 g and 30 g HC interventions, respectively. Compared with 0 g HC, the PINP concentration × time total AUC (Figure 3B) was 1.4 times greater in the 15 g HC intervention ($P = 0.024$), and 1.8 times greater in the 30 g HC intervention ($P < 0.001$). Finally, the AUC was 1.2-fold greater in the 30 g compared with the 15 g HC intervention ($P = 0.033$).

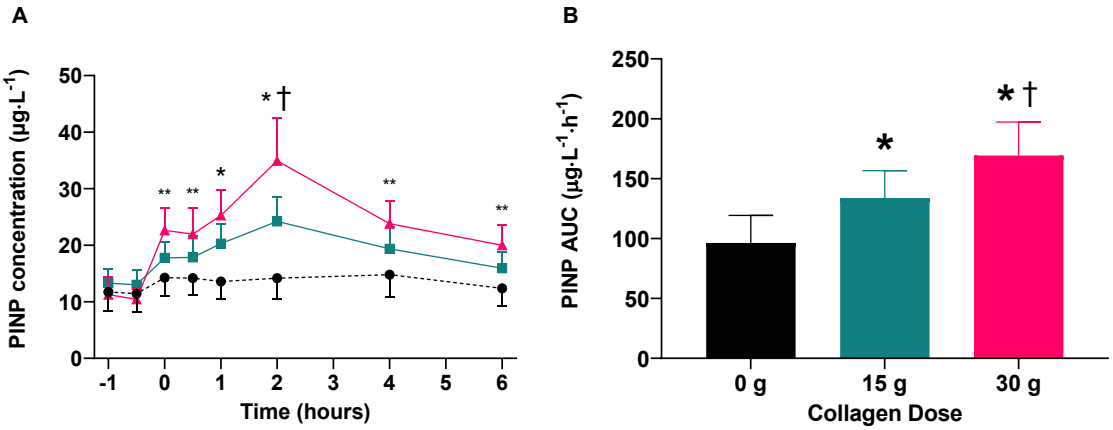


**Figure 3**. **A**. Mean ± SEM serum PINP concentration over time (n = 8). Supplementation was provided at exercise -1 h (0 g black circles, 15 g green squares, 30 g pink triangles). Resistance exercise was completed between 0 h and +0.5 h; * 15 g and 30 g greater than 0 g; † 30 g greater than 15 g; ** 30g, not 15 g, greater than 0g. **B.** The serum PINP concentration × time total area under the curve (AUC) in response to resistance exercise combined with 0 g, 15 g, and 30 g hydrolysed collagen supplementation (n = 8). Data are mean ± SEM. * Greater than 0 g, † 30 g greater than 15 g ($P < 0.05$).

***Effects of collagen supplementation combined with resistance exercise on collagen breakdown***

Plasma β-CTx concentration did not differ at baseline between the three interventions ($P < 0.0001$, Figure 4). There was a main effect of time ($F_{1,8} = 16.4$, $P = 0.004$), i.e. during all three interventions, plasma β-CTx decreased immediately after RE and remained lower for the duration of the interventions. Additionally, at 6 h post-exercise (the final sample time point in each intervention), β-CTx concentration was greater than at +0.5 h ($P < 0.001$) and +2 h ($P < 0.001$), but lower than baseline ($P < 0.001$). There was no main effect of dose ($F_{2,13} = 0.911$, $P = 0.421$), and there was no dose × time interaction ($F_{3, 24} = 0.675$, $P = 0.595$) on plasma β-CTx concentration. Finally,

there was no difference in the β-CTx concentration × time total AUC between interventions ($F_{2,14}$ = 1.24, $P$ = 0.320; Figure 4B).

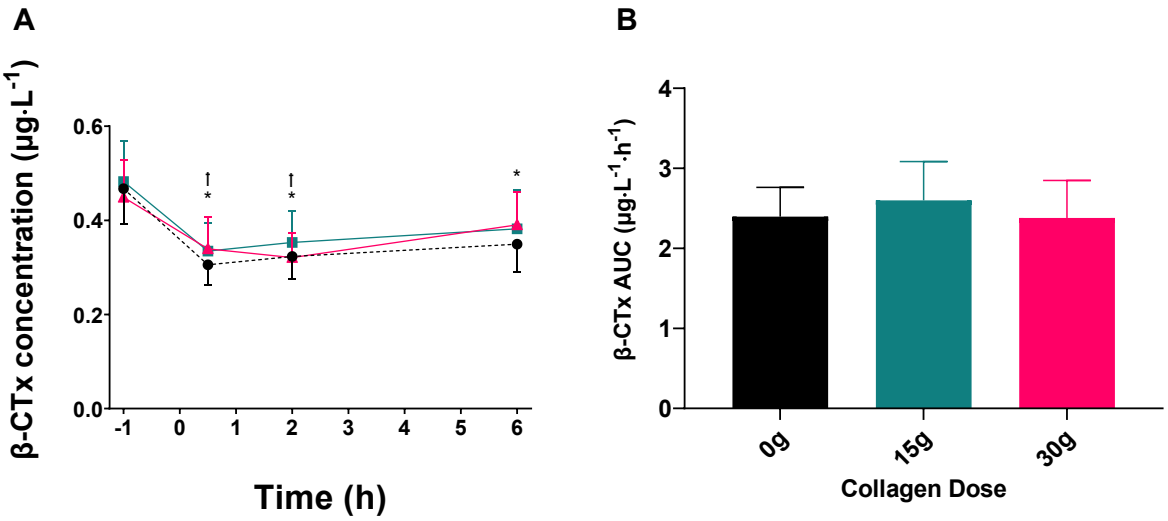


**Figure 4**. **A**. Mean ± SEM plasma β-CTx concentration over time (n = 8). Supplementation was provided at exercise -1 h (0 g black circles, 15 g green squares, 30 g pink triangles). Resistance exercise was completed between 0 h and +0.5 h; * lower than -1 h; † lower than -1h and +6h ($P < 0.05$). **B**. Plasma β-CTx concentration × time total area under the curve (AUC) in response to resistance exercise combined with 0 g, 15 g, or 30 g hydrolysed collagen supplementation (n = 8).

***Serum Amino Acid Profile***

Figure 5 displays the serum concentrations of 18 amino acids that constitute type I collagen over the 7 h period after ingestion of 0, 15, or 30 g HC. There were main effects of time for glycine, proline, hydroxyproline, glutamic acid, alanine, arginine, lysine, serine, leucine, valine, phenylalanine, threonine, isoleucine, tyrosine and methionine ($P < 0.05$). There were main effects of dose for glycine, proline, hydroxyproline, alanine, arginine, lysine, serine, leucine, valine and isoleucine, with 15 and 30 g HC displaying greater concentrations of these amino acids than 0 g HC

($P < 0.05$), and 30 g HC displaying greater concentrations than 15 g HC ($P < 0.05$). There were dose × time interactions for all but 3 amino acids (aspartic acid, histidine, and glutamine).

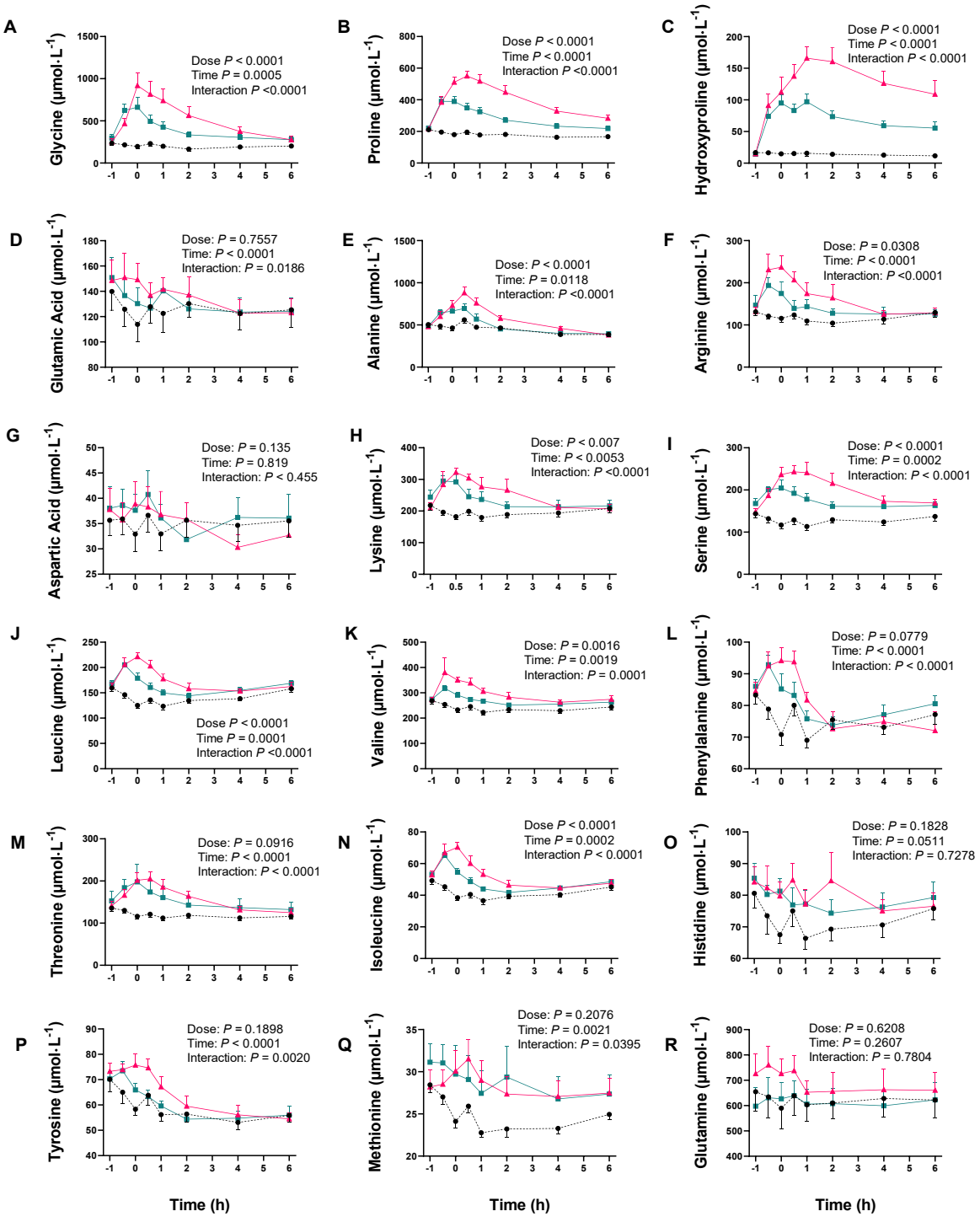

**Figure 5**. Mean ± SEM serum concentration of 18 amino acids (glycine, A; proline, B; hydroxyproline, C; glutamic acid, D; alanine, E; arginine, F; aspartic acid, G; lysine, H; serine, I; leucine, J; valine, K; phenylalanine, L; threonine, M; isoleucine, N; histidine, O; tyrosine, P; methionine, Q; and glutamine, R) across time after ingestion of 0 g (black circles), 15 g (green squares) and 30 g (pink triangles) of hydrolysed collagen. Hydrolysed collagen ingestion took place at -1 h, and resistance exercise began at 0 h.

## 4.4 Discussion

This is the first study to demonstrate that whole body collagen synthesis increases in response to resistance exercise (RE) in combination with the ingestion of vitamin C-enriched hydrolysed collagen (HC) in resistance-trained, *middle-aged* men. Our data support our hypothesis, as the nutritional supplementation (both 15 and 30 g hydrolysed collagen) was sufficient to increase serum PINP concentration, and the PINP concentration × time AUC more than RE alone in a 6 h period post RE. Interestingly, the 30 g HC intervention led to a ~27 % greater PINP total AUC compared to the 15 g HC intervention, which led to a ~39 % greater total AUC than the 0 g HC intervention. This HC dose-response effect on whole body collagen synthesis was reflected by a similar HC dose-response effect on serum collagen amino acid availability. These data therefore show that high-intensity lower-limb RE alone is insufficient to stimulate collagen synthesis in resistance-trained, middle-aged men. However, supplementing RE in this population with either 15 or 30 g HC (enriched with vitamin C) rescues this apparent anabolic resistance to RE, probably by collagen amino acids stimulating collagen synthesis independently of RE.

In our study design, we chose to use *high intensity* leg press RE to stimulate the quadriceps muscle-tendon unit (MTU), as this mode of RE is considered a primary stimulus to enhance the material and mechanical properties of human patellar tendon (Bohm *et al.*, 2015). There are no other studies, to our knowledge, that have described the collagen synthesis response to RE in *middle aged* men. Surprisingly, we observed no increase in collagen synthesis following RE alone, which was unexpected given various types of exercise, including running, skipping, and RE have all been shown to increase collagen synthesis in young men and women independently of nutritional supplementation (Langberg *et al.*, 1999a; Miller *et al.*, 2007; Shaw *et al.*, 2017; Lee *et al.*, 2023b). In the time since our study was initiated, 12 high intensity sets of RE have been shown to increase the fractional synthetic rate (FSR) of muscle connective tissue protein in older men (Holwerda & van Loon, 2022). Furthermore, the collagen FSR of older tendon has very recently been shown to increase after 4 weeks' moderate intensity leg press RE using 12 weekly sets of leg-press RE (Quinlan *et al.*, 2021). Taken together these findings imply that a greater volume (i.e. greater number of sets) may be required to maximize the serum PINP response in *middle-aged* men. However, Lee et al. (Lee *et al.*, 2023b) observed an increase in whole body collagen synthesis (also measured via serum PINP concentration) following RE alone in *young* men, using a very similar RE protocol to that used in our study. Thus, our data suggest a blunted collagen synthesis response to RE alone in *middle-aged* men for a given volume and relative intensity of RE. Nevertheless, the addition of a HC supplement (containing at least 15 g HC) appears to 'rescue' this blunted collagen synthesis to RE in this population.

There is a parallel body of research describing the interaction between RE, dietary protein supplementation and muscle protein synthesis (MPS) responses (Phillips,

2016). The term ‘anabolic resistance’ describes how older skeletal muscle is not as responsive as young muscle to RE (Kumar *et al.*, 2009). The provision of additional whey protein can assist in mitigating this issue and recovering MPS rates after RE in older tissues, but the dose required can be double that which maximizes MPS in young (Yang *et al.*, 2012). At least 15 g collagen supplementation rescued the collagen synthesis response in our study, and the advantage observed during the 30 g intervention may be indicative of a need for higher doses as age increases. This is likely given that in young men, collagen synthesis increased after RE alone and increased further with ingestion of 30 g HC but with no benefit of a 15 g dose (Lee *et al.*, 2023b). It is interesting that just 15 g HC was sufficient to augment collagen synthesis in *middle-aged* men, which suggests that the collagen synthesis response may already be close to maximized by RE alone in younger populations. Furthermore, ageing is associated with reduced collagen synthesis (at rest) in men (Kehlet *et al.*, 2018b), which may help explain the importance of exogenous collagen supplementation, even in relatively small amounts, to augment collagen synthesis in *middle-aged* men following RE. It is noteworthy, however, that peak serum PINP concentration (which occurred after RE) and total PINP AUC were lower in *middle-aged* men, and more comparable with the *pre-exercise* levels observed in young men (Lee *et al.*, 2023b), further suggesting a blunted response of older connective tissue to collagen ingestion and RE. It remains to be seen whether ingestion of >30 g HC would further recover the post RE collagen synthesis response in middle-aged men.

Compared to fasted levels in the current study, there was a 2 – 2.9-fold increase in peak PINP concentration following RE with HC ingestion. Additionally, we have shown that collagen feeding alone increases collagen synthesis, albeit for a short duration and to a much lesser extent than during the post RE period. The increases in

serum PINP concentration in response to both exogenous collagen supplementation and RE correspond to the dose-response elevation (30 > 15 > 0 g) in serum amino acid concentration (Figure 5). The key amino acids found in collagen (e.g. glycine, proline, and hydroxyproline) peaked in our serum samples between 1 and 2 h after ingestion of the supplement (Figure 5**)**, which is in agreement with previous studies in young healthy men and women (Shaw *et al.*, 2017; Alcock *et al.*, 2019b; Aussieker *et al.*, 2023; Lee *et al.*, 2023b; Lee *et al.*, 2024b). There are several mechanisms by which exogenous collagen ingestion may have promoted collagen synthesis. Firstly, while glycine is a non-essential amino acid, the amount synthesized per day in humans may be insufficient for optimal collagen synthesis (Meléndez-Hevia *et al.*, 2009), and the abundance of dietary glycine supplied by collagen supplements (and confirmed by our amino acid profiles) may make up for this shortcoming. Secondly, in a bovine model, 10 g/day of dietary glycine increased collagen synthesis in articular chondrocytes, which are specialized cells in the ECM of cartilage (de Paz-Lugo *et al.*, 2018), suggesting a collagen synthesis-stimulating role of glycine. Additionally, proline and hydroxyproline have been shown *in vitro* to regulate TGF-β (transforming growth factor-β) in fibroblasts, leading to downstream phosphorylation of protein kinase B (Akt) and mammalian target of rapamycin complex I (mTORCI), the key regulatory pathway leading to collagen protein synthesis (Surazynski *et al.*, 2010). Due to the greater post-exercise responses in our data (compared to the pre-exercise postprandial period), it is conceivable that the increased levels of collagen synthesis in the 15 g and 30 g HC interventions were the result of cumulative signalling from both the RE induced mechanical loading (Mousavizadeh *et al.*, 2020) and amino acid induced stimulation of growth factors (Surazynski *et al.*, 2010) within the ECM of target connective tissue (e.g. in the tendon and muscle).

We measured plasma β-CTx concentration as a marker of whole-body collagen breakdown, and observed a sustained decrease after supplement ingestion (regardless of HC dose) and RE, suggesting a positive net collagen turnover following RE with or without HC ingestion. In a recent study, Aussieker and colleagues (Aussieker *et al.*, 2023) found that ingestion of both 30 g whey protein and 30 g HC with RE appeared to inhibit whole body collagen breakdown (i.e. serum CTX-I concentration decreased) 2 h after RE in young adults. However, this was not the case when water alone was ingested with RE, where serum CTX-I concentration remained unaltered throughout the intervention. This may suggest a positive effect of protein ingestion (regardless of protein type) on whole body collagen breakdown were it not for the fact that plasma β-CTx concentration decreased in our 0 g HC intervention. In our study, we calorie matched both the 0 g and 15 g HC beverages to the energy of the 30 g HC supplement, thus all of our RE interventions took place with the same energy availability. It is likely, therefore, that the inhibited collagen breakdown observed in young adults (Aussieker *et al.*, 2023), and in our study of middle-aged men, was the result of calorie intake, regardless of macronutrient composition, which is in line with previous work by Henriksen *et al.* (2003). Furthermore, given that plasma β-CTx concentration decreased 90 min after supplement ingestion (time: 08:30) in our study, and remained lower than fasted concentrations (time: 07:00) for the remainder of all three interventions (Figure 4), it is unlikely that the decrease we observed in plasma β-CTx concentration was associated with diurnal variation, as previously suggested (Qvist *et al.*, 2002).

It has previously been shown that procollagen type I C-terminal propeptide concentration (another marker of collagen synthesis) and Achilles tendon collagen content (estimated from echo intensity) simultaneously increased after two months'

resistance training in healthy young men, which corresponded to an increase in Achilles tendon stiffness (Kubo *et al.*, 2012). This suggests that enhanced tendon collagen synthesis and content are required to improve the mechanical properties of the MTU in response to progressive overload. Thus, given our findings, we can speculate that repeated stimulation (through RE and 30 g HC supplementation) would result in greater increases in tendon size and stiffness in *middle-aged* men over a prolonged period. Emerging data in young men (Jerger *et al.*, 2022; Jerger *et al.*, 2023) and young women (Lee *et al.*, 2023a) suggest positive outcomes in certain morphological and mechanical tendon properties when HC is ingested in combination with resistance training in young populations. However, it is not yet known if similar outcomes would be found in *middle-aged* men and women. One previous study, however, did show that daily supplementation with 10 g collagen for 12 weeks did not increase serum PINP concentration in middle-aged men (Bongers *et al.*, 2020). However, it is unclear if increasing the dose of collagen and/or supplementing in combination with RE would alter this response.

*Limitations*

Human tendon (Langberg *et al.*, 1999a) and serum (Huang *et al.*, 2022b) PINP concentration are known to increase after an acute bout of exercise, and serum PINP remains elevated for up to four days after RE in humans (Virtanen *et al.*, 1993), which is in accordance with the elevated collagen fractional synthetic rate in skeletal muscle and tendon following RE (Miller *et al.*, 2005b). Thus, although we did not measure collagen synthesis directly within the MTU, measuring a biomarker of collagen synthesis in the form of PINP, which is cleaved off during the maturation of procollagen to collagen (Heinemeier *et al.*, 2016), is a reliable alternative. Finally, this study included solely male participants and, considering the potential effects of

oestrogen on collagen synthesis (Hansen, 2018), it is not known if we would have seen different results in resistance-trained, middle-aged, pre- or postmenopausal women.

*Conclusion*

Our data show that the combination of high-intensity, lower-limb resistance exercise and hydrolysed collagen supplementation increases collagen synthesis in middle-aged, resistance-trained men, in a dose-response manner, i.e. 30 g > 15 g > 0 g. Crucially, this important finding was reflected in a collagen dose-response effect on the bioavailability of collagen amino acids known to independently stimulate collagen synthesis. Given the lack of response to resistance exercise alone, our findings suggest the sensitivity of collagen rich tissues, such as tendon and ligament, to resistance exercise may be reduced in middle-age, which can be recovered with the ingestion of hydrolysed collagen.

# Chapter Five

# The effect of hydrolysed collagen supplementation on markers of collagen turnover following a single bout of resistance exercise in resistance-trained, pre-menopausal, middle-aged women: a case study

## Prelude

Chapter Three focused on the habitual dietary intake of collagen in Irish adults. Although a key finding was that collagen intake comprised only 4% of total protein intake (~4 $g \cdot day^{-1}$ in men), collagen intake was even lower in women (~3 $g \cdot day^{-1}$), regardless of age. Chapter Four investigated the effect of 0 g vs. 15 g vs. 30 g HC ingested prior to high-intensity leg press resistance exercise on biomarkers of collagen turnover in resistance-trained, middle-aged men. The main outcomes were that RE alone did not increase collagen synthesis in middle-aged men, but 30 g HC was the optimal dose to recover the collagen synthesis response. It has recently been shown that the late follicular phase (associated with the highest serum oestrogen concentration) of the menstrual cycle (MC) results in a lower collagen synthesis response to RE and 30 g HC ingestion in a young female athlete. Therefore, this chapter examined the effects of an acute bout of high intensity leg press RE with 0 g or 30 g HC in two resistance-trained, premenopausal, middle-aged women during the late follicular phase of their MC. This study was performed between November 2019 and March 2020.

# Abstract

This case study examined the effect of hydrolysed collagen (HC) supplementation on the collagen turnover response to resistance exercise (RE) in middle-aged, premenopausal, resistance trained women. Two participants (Participant 1, 43 years; 160.5 cm, 63.5 kg; ingesting 0 g HC and Participant 2, 42 years; 172.6 cm, 69.4 kg; ingesting 30 g HC) ingested either 0 g or 30 g of HC 1 h prior to completing four sets of 10-repetition maximum leg press RE during the late follicular phase of their menstrual cycles. Serum and plasma biomarkers of collagen turnover were examined, including procollagen type I N-terminal propeptide (PINP; collagen synthesis) and β-isomerised C-terminal telopeptide (β-CTX; collagen breakdown), along with 18 collagen amino acids. Participant 2 (30 g HC) exhibited a 2-fold peak increase in serum PINP concentration following RE, with a 2.3-fold greater concentration × time area under the curve (AUC) compared to Participant 1 (0 g HC), whose serum PINP concentration remained similar to baseline throughout the intervention. Plasma β-CTX concentration decreased immediately after RE in both participants and returned to baseline within 6 hours in Participant 2 (30 g HC) but remained suppressed in Participant 1 (0 g HC). Serum glycine, proline, and hydroxyproline concentrations increased following ingestion of 30 g HC (Participant 2), but not after ingestion of 0 g HC (Participant 1). Thus, RE alone was not associated with an increase in collagen synthesis during the late follicular phase of the menstrual cycle, suggesting either an inhibitory effect of oestrogen on collagen synthesis and/or connective tissue anabolic resistance to RE in middle-aged, premenopausal women. However, ingestion of 30 g HC prior to RE appeared to rescue the collagen synthesis response, thus providing a strategy to overcome any inhibitory effects of oestrogen and/or ageing on collagen synthesis following RE in middle-aged, resistance trained women.

# 5.1 Introduction

Female athletes have a higher risk of soft tissue (including collagenous tissues, such as skeletal muscle, tendon and ligament) injuries than their male counterparts (Hewett *et al.*, 2016). In addition to muscle weakness (Augustsson & Ageberg, 2017), high circulating oestrogen levels, such as those present in healthy, naturally menstruating women, may influence the mechanical properties of tendon and ligament and thus injury risk (Hansen & Kjaer, 2014). Specifically, the late follicular phase of the menstrual cycle (MC), when circulating oestrogen is at its highest concentration, is associated with greater joint laxity (Park *et al.*, 2009) and injury risk (Herzberg *et al.*, 2017). Since skeletal muscle relies on tendon and ligament for effective force transfer, any influence of oestrogen on passive connective tissue will indirectly impact muscle function and performance (Hansen & Kjaer, 2014).

This poses a particular issue for middle-aged women, a demographic in which the incidence of tendinopathy peaks (Clayton & Court-Brown, 2008b). Middle-age represents a complex transitional period, with women spanning pre-, peri-, and post-menopausal states (Su and Freeman, 2009), which may impact collagen synthesis, tissue adaptability, and injury risk. For example, post-menopausal women using hormone replacement therapy (HRT) exhibit higher tendon collagen synthesis rates than non-HRT users (Hansen *et al.*, 2009a), whereas young women using oral contraceptive pills (OCP) show reduced tendon collagen synthesis compared to non-OC users (Hansen *et al.*, 2009c). Such hormonal variability complicates research design and suggests the need for tailored training and nutrition recommendations across various sub-groups of middle-aged women.

Collagen-rich tissues such as tendons, ligaments and bone express oestrogen receptors (Ciana *et al.*, 2003). Accordingly, women exhibit lower tendon collagen fractional synthetic rate at rest and in response to exercise compared to men, suggesting reduced connective tissue adaptability (Miller *et al.*, 2007). Although the relative gains in muscle strength and size occur similarly between the sexes following resistance training (RT) (Van Every *et al.*, 2024), increases in tendon size and stiffness appear attenuated in females, compared to age-matched males (Onambele-Pearson & Pearson, 2012; McMahon *et al.*, 2018). Despite this, RT remains the principal method for reducing injury risk (Lauersen *et al.*, 2018) and improving athletic performance (Van Hooren *et al.*, 2024).

Collagen supplementation has been suggested as a potential method for enhancing tendon adaptation to training and maintaining connective tissue health (Shaw *et al.*, 2017; Shaw *et al.*, 2019), and emerging data support the use of HC with RT for long-term tendon remodelling in young female athletes (Lee *et al.*, 2023a; Lee *et al.*, 2024a). In healthy young men, it was recently demonstrated that 30 g HC enhances the collagen synthesis response to RE (Lee *et al.*, 2023c). A follow-up case study in a resistance-trained female athlete suggested 30 g HC ingestion augments RE-induced collagen synthesis. Despite this, the collagen synthesis response to both RE alone, and RE with HC was attenuated in the late follicular phase, suggesting an inhibiting effect of oestrogen (Lee *et al.*, 2024c).

In addition, the findings of Chapter Four suggest that RE alone may not be sufficient to increase collagen synthesis in middle-aged trainees, but that ingestion of 30 g HC can recover the collagen synthesis response to RE (more so than ingestion of 15 g HC). Despite these apparent effects of age and sex on the collagen synthesis response to RE and HC ingestion, the effects in middle-aged premenopausal women are yet to

be investigated. Therefore, the main objective of this study was to describe how systemic markers of collagen turnover respond to RE with and without 30 g HC ingestion, in two pre-menopausal middle-aged athletes during the late follicular phase of their respective menstrual cycles. Despite the lack of generalisability, it is envisaged that this case study will provide an important step in bridging the female data gap and generating hypotheses for future work.

# 5.2 Method

***Participants***

Two resistance-trained, middle-aged women volunteered to take part in this case study. Both were naturally menstruating at the time of recruitment and for the duration of the study, and neither participant had history of oral contraceptive use or alternatives. 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). Neither participant had a history of tendinopathy and both participants were injury free for at least six months prior to participation.

***Research Design***

This was a double-blind case study, where neither the researcher nor the participants were aware of which supplement was ingested. Following recruitment, both participants were asked to track the course of their menstrual period for at least two consecutive cycles using a commercially available calendar application (Flo, Flo Health UK LTD, London, UK). During this time, participants were familiarised to the RE and measurement techniques used in this study, and their leg press (Samson, New

Mexico, USA) 10-repetition maximum (10-RM) was assessed. To ensure consistency in baseline measurements, participants were asked to report to the laboratory for assessment of muscle strength on the first day of menses. This timing was chosen to standardise the hormonal status of the participants, as the first day of menses represents a low point in circulating oestrogen levels. Each participant returned to the laboratory on day 14 of their respective menstrual cycle, which represented the day of highest circulating oestrogen concentration. Upon arrival to the laboratory, Participant 1 was provided with a placebo drink (0 g), and Participant 2 was provided with a supplement containing 30 g of hydrolysed collagen (30 g). Both supplements were matched for energy intake and vitamin C content (50 mg per bolus), as vitamin C is an essential co-factor in the biosynthesis of collagen synthesis (Canty & Kadler, 2005). One hour following ingestion, each participant completed four sets of 10 repetitions' leg press exercise at their respective 10-RM load. Venous blood samples were collected at regular intervals for the duration of each trial, and serum/plasma samples were analysed for PINP concentration, amino acid content, and β-isomerized C-terminal telopeptides (β-CTX, a marker of collagen breakdown). The study received approval from Liverpool John Moores University's Research Ethics Committee (Approval number: 19SPS049) and complied with the Declaration of Helsinki. Both participants provided written informed consent prior to taking part in this study.

***Measurement of isometric strength and 10-RM leg press***

Participants were firmly and securely strapped to an isokinetic dynamometer (Biodex System 3, IPRS, Suffolk, UK) in a seated position with their knee joint set at 90° knee flexion and their hip joint set at 85°. While participants were seated on the dynamometer, they performed a specific warm up for isometric testing, comprising 10 repetitions of isokinetic knee extension and flexion performed at $60°/s^{-1}$. During this

set, participants gradually increased their effort, beginning with 10% of their perceived maximum effort and increasing their effort by 10% on every repetition before a final 10$^{th}$ repetition performed at maximal (100%) effort. Participants then performed three submaximal isometric knee extension/flexions at 50 – 80 % perceived effort. This was followed by 2- 3 maximal isometric knee extensions and three maximal isometric knee flexions lasting 2- 3 s each, with 60 s rest between each maximal effort.

Participants performed a brief dynamic warm-up comprising 5 min submaximal cycling and 5 min dynamic stretching before performing two to four sets of the leg press exercise using the empty sled i.e., the lowest usable weight on the machine, which weighed 40 kg. This was done to determine each participant's unique foot placement and range of motion on the machine. Participants then performed an incremental loading protocol to determine their 10-RM. Firstly, the performed one to two sets of five to ten repetitions at an estimated 50 to 70 % of their estimated 10-RM. Thereafter, the load was increased by 5 to 20 kg on each successive set for a total of four to six attempts, with 3 min rest between each attempt, until the individual could only complete ten repetitions or less. The 10-RM was the load used in the final set where 10 repetitions were successfully completed.

***Supplementation, resistance exercise and blood sampling***

Upon arrival to the laboratory at 07:00 am following a 10 h overnight fast, participants were fitted with an indwelling cannula in the antecubital vein to permit repeated 6 mL blood sampling at multiple time-points over the subsequent 7 h period. Immediately after the baseline blood sample was taken, Participant 1 was provided with a beverage (0 g HC) containing 30.5 g maltodextrin (MyProtein, Manchester, UK), 50 mg vitamin C powder (Holland and Barrett, Dublin, Ireland) and 3 g non-caloric sweetener (Pure

Via 100% Xylitol, Mersiant UK Ltd, Buckinghamshire, UK). Participant 2 was provided with a collagen supplement (30 g HC) containing 30 g unflavoured hydrolysed collagen protein (Collagen Protein, MyProtein, Manchester, UK), 50 mg vitamin C powder (Holland and Barrett, Dublin, Ireland) and 3 g non-caloric sweetener (Pure Via 100% Xylitol, Mersiant UK Ltd, Buckinghamshire, UK), and both beverages were matched for energy and taste. One hour after ingestion, the participants performed four sets of 10 repetitions at their previously determined 10-RM load on the leg press. Throughout the trial, blood was drawn at the following time points, where -1 h represents arrival at the laboratory, 0 h represents the time immediately prior to starting RE: -1h, -0.5h, 0h, +0.5h, +1h, +2h, +4h, +6h. This was followed by centrifugation, separation into serum and plasma, and storage at −70°C.

***Measurement of PINP, β-CTX and Amino Acid profile***

The detailed methods for analysis of blood samples are described in Chapter Four. In brief, blood markers of collagen synthesis (serum PINP concentration) and breakdown (EDTA plasma β-CTX concentration) determined from duplicate samples using a competitive enzyme-linked immunosorbent assay (ELISA) (Cloud-Clone Corp, Wuhan, China) and electrochemiluminescence immunoassay (ECLIA) on a Cobas e601 analyser (Roche Diagnostics, Germany). Additionally, 18 amino acids related to collagen composition were measured simultaneously using anionic ion-pair reverse phase liquid chromatography tandem mass spectrometry (LC-MS/MS) system following derivatisation of the amino acid with *n*-butanol hydrogen chloride.

# 5.3 Results

***Lower body strength characteristics***

Both participants displayed similar lower-body strength characteristics which are described in Table 1. The difference in mean maximal voluntary knee extension (KE) torque between participants was 51 N·m, and the difference 10 RM leg press between participants was 20 kg.

**Table 1**. Lower body strength characteristics of middle-aged premenopausal athletes (n = 2)

| | **Participant 1** | **Participant 2** |
|---|---|---|
| **KE MVT (N·m)** | 205 | 256 |
| **KF MVT (N·m)** | 89 | 109 |
| **10 RM Leg Press (kg)** | 120 | 140 |

*N·m,* Newton metres; $mm^2$: millimetres squared; *KE MVT,* Knee Extension Maximum Voluntary Torque; *KF MVT,* Knee Flexion Maximum Voluntary Torque; *10 RM,* Ten Repetition Maximum.

***Effect of collagen supplementation combined with resistance exercise on collagen synthesis in women***

There were minor differences in serum PINP concentration at baseline between participants (Participant 1: 11.4 $\mu \cdot L^{-1}$).; Participant 2: 18.1 $\mu \cdot L^{-1}$). In Participant 1 (0 g HC), serum PINP concentration remained similar to baseline throughout the trial, apart from two specific time points: 30 min post ingestion, and 6-h post-RE, where PINP concentration was 1.6-fold higher than baseline values. Serum PINP concentration

peaked at 4 h post-RE in Participant 2 (30 g HC), which was 2-fold greater than their baseline concentration, and 3.4-fold greater than that of Participant 1 (0 g) at the same time point. Immediately following RE in Participant 1 (0 g HC), serum PINP concentration returned to baseline levels and remained stable for the subsequent four hours. The serum PINP concentration × time AUC in Participant 2 (30 g HC) was 2.3-fold greater than that of Participant 1 (0 g HC) (Figure 1).

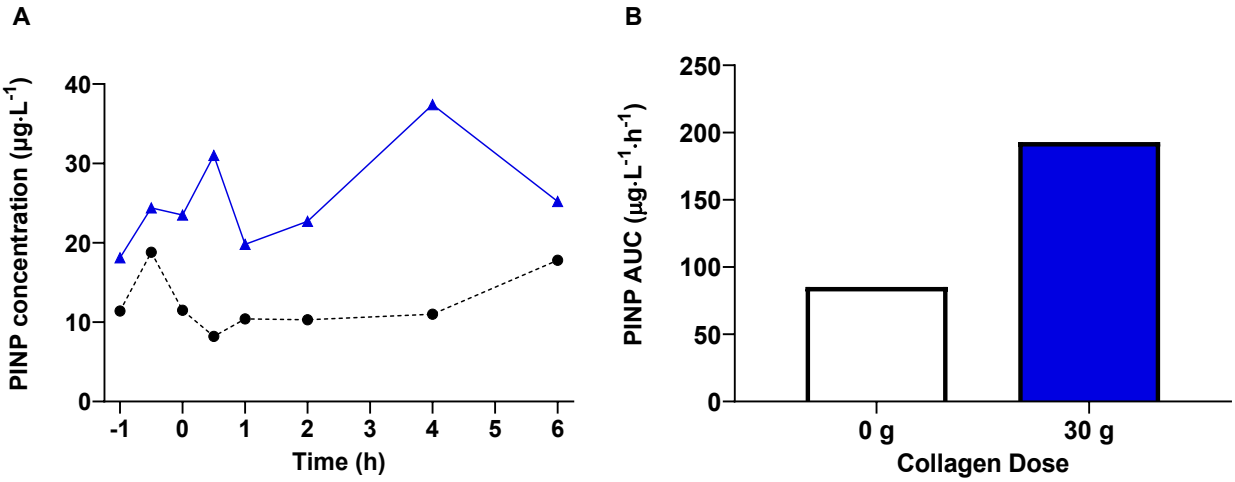


**Figure 1. A.** Serum PINP concentration in response to leg press resistance exercise combined with either a placebo containing 0 g hydrolysed collagen (Participant 1, black circles) or 30 g hydrolysed collagen (Participant 2, blue triangles) in two middle-aged women. **B.** Between subject (n=2) concentration × time total area under the curve (AUC) for PINP response to resistance exercise combined with collagen supplementation in middle-aged pre-menopausal women during the late follicular phase of menstrual cycle. Participant 1 (0g HC, white bar) received the placebo, while Participant 2 (30g HC, blue bar) received 30g hydrolysed collagen.

***Effect of collagen supplementation combined with resistance exercise on collagen degradation in women***

Plasms β-CTX levels in Participant 2 (30 g HC) were consistently higher than in Participant 1 (0 g HC), with baseline levels of 0.41 $\mu g \cdot L^{-1}$ and 0.31 $\mu g \cdot L^{-1}$, respectively. Participant 1 and 2 experienced a 16% and 20% decrease in plasma β-CTX concentration, respectively, immediately after RE. In Participant 1 (0 g HC), plasma β-CTX concentration decreased and remained low for the duration of the intervention (further decreasing to 0.22 $\mu g \cdot L^{-1}$ at 6 hours post-RE). In contrast, plasma β-CTX concentration returned to baseline levels (0.41 $\mu g \cdot L^{-1}$) by 6 hours post-RE in Participant 2 (30 g HC). The plasma β-CTX concentration × time area under the curve (AUC) was 1.8 $\mu \cdot L^{-1} \cdot h^{-1}$ for Participant 1 and 2.6 $\mu \cdot L^{-1} \cdot h^{-1}$ for Participant 2, representing a 1.4-fold greater overall collagen breakdown in the 30 g compared to the 0 g HC participant (Figure 2).

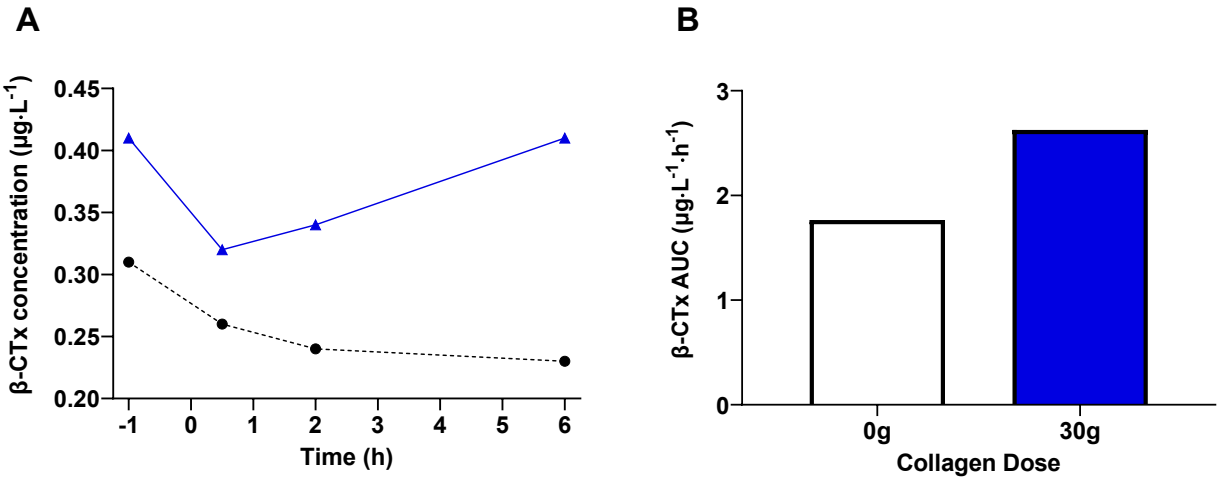


**Figure 2. A.** Plasma β-CTX concentration in response to leg press resistance exercise combined with either a placebo containing 0 g hydrolysed collagen (participant 1, black circles) or 30 g hydrolysed collagen (participant 2, blue triangles) in two middle-aged women. **B.** Between subject (n = 2) concentration × time total area under the curve (AUC) for β-CTX response to resistance exercise combined with collagen supplementation in middle-aged pre-menopausal women during the late follicular phase of menstrual cycle. Participant 1 (0g HC, white bar) received the placebo while participant 2 (30g HC, blue bar) received 30g of hydrolysed collagen

***Serum amino acid profile***

Figure 3 displays the serum concentration of 18 amino acids that constitute type I collagen over the 7 h period after ingestion of 0 or 30 g HC in Participant 1 and 2, respectively. The peak concentration and for the key collagen amino acids, i.e. glycine, proline, and hydroxyproline, was higher in Participant 2 (30 g), while there was no change in these amino acids across time in Participant 1 (0 g).

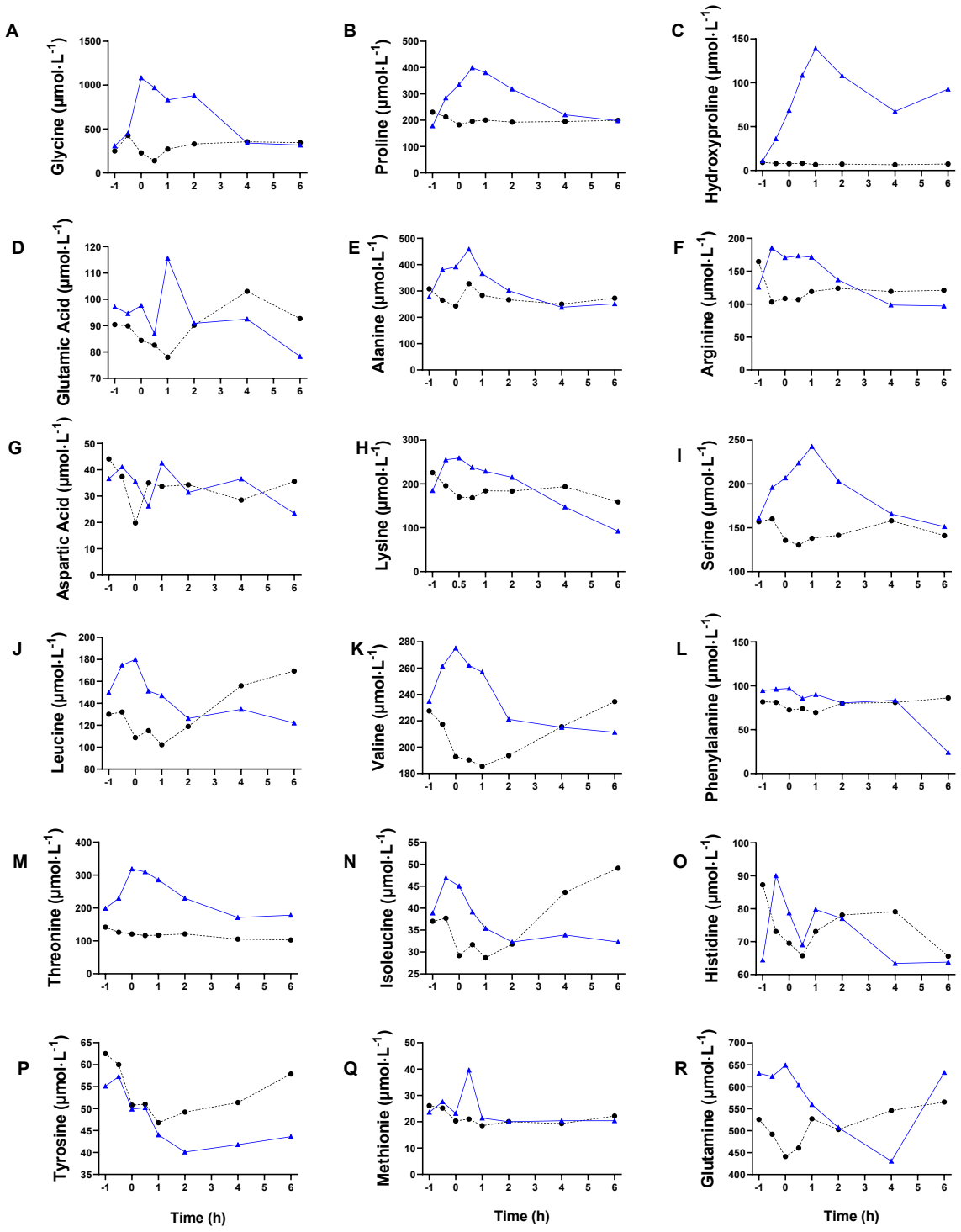


**Figure 3.** Serum amino acid concentration across time after ingestion of 0 g (black dashed line) and 30 g (blue line) hydrolysed collagen. Hydrolysed collagen ingestion occurred at -1 h, and resistance exercise began at 0 h.

## 5.4 Discussion

The present case study is the first to describe the effects of RE with and without hydrolysed collagen supplementation on whole body collagen synthesis in healthy, naturally menstruating, middle-aged, premenopausal (resistance trained) women. The main observation was that in the late follicular phase of the menstrual cycle, RE alone (0 g HC) did not increase serum PINP concentration i.e., collagen synthesis was unaffected. However, the participant who ingested 30 g HC prior to RE experienced an increase in serum PINP concentration post-RE, and more than double the concentration × time area under the curve (AUC) compared to the participant who ingested 0 g HC. Overall, the change in serum markers of collagen synthesis following RE and HC were similar to those we reported for middle-aged, resistance trained men in Chapter Three. These findings indicate that the collagen synthesis response to RE may be attenuated (either as a consequence of high oestrogen concentration or ageing, or both) in middle-aged, premenopausal women, although this may be overcome by ingestion of 30 g HC.

At baseline i.e., after an overnight fast and before HC ingestion, serum PINP in both participants was similar to that observed in middle-aged men (Chapter Four) and the 36-year-old female athlete described in the case study by Lee *et al.* (2024c). However, post-RE changes in PINP concentration were markedly different between participants. The most striking observation was that there was no obvious peak in serum PINP concentration following heavy RE alone (0 g HC), which is in contrast to findings in young men and women (Miller *et al.*, 2007; Lee *et al.*, 2023c; Lee *et al.*, 2024c). Given that our data collection took place in the late follicular phase of each participants respective menstrual cycles, it could be speculated that the lack of response to RE

alone (0 g HC) was due to a possible inhibitory effect of oestrogen on collagen synthesis (Miller *et al.*, 2007; Hansen *et al.*, 2009a; Lee *et al.*, 2024c). However, circulating oestradiol is known to fluctuate substantially (i.e. non predictive fluctuations, outside of those expected across the phases of the MC) in the late reproductive years, and may trend downwards as proximity to menopause approaches (Burger *et al.*, 1999). As such, middle-aged premenopausal women, such as those in our case-study, may not experience the same inhibiting effects of oestrogen on collagen synthesis as described in young naturally menstruating women (Miller *et al.*, 2007; Hansen *et al.*, 2009a; Lee *et al.*, 2024c). Unfortunately, the lack of circulating oestradiol measurement in our participants precludes us from drawing strong conclusions, however given the lack of PINP response to RE alone in middle-aged men in Chapter Four, we can speculate that the blunted collagen synthesis may simply be due to the age of our participants.

Although serum PINP concentrations remained similar to baseline for the duration in the 0 g HC intervention, Participant 1 (0 g HC) showed a small increase at 6 h post RE (+ 6.8 $\mu \cdot L^{-1}$ compared to 4 h post RE). Miller et al. (2007) documented that mechanical loading increased serum PINP concentration in young women, peaking at 72 h post-exercise. Therefore, at the latest time point in our study, we may be observing the beginning of a delayed response. This is highly speculative however, since the PINP increased at 6 h post-RE was minimal, and the absolute concentration was similar to baseline, thus it may simply represent a transient rise, or potentially, diurnal variation. It remains unclear how repeating interventions during the onset of menses might influence PINP, but with circulating oestrogen expected to be lower, there may have been an amplified PINP response to RE in both participants (Lee *et al.*, 2024c). Moreover, differences between the leg-kicking model by Miller and colleagues (2007)

and our high-load RE model could influence collagen synthesis timing. As we are the first to report the collagen synthesis response to acute, high-intensity lower-limb RE in middle-aged women, our data tentatively suggest that PINP responses peak within the initial hours post-RE.

In contrast, peak PINP concentration was double the baseline concentration in Participant 2 (30 g HC), and the PINP concentration × time AUC in Participant 2 was over just over double that of Participant 1 (0 g HC). This was consistent with the increases in serum glycine, proline, and hydroxyproline following 30 g HC ingestion and mirrors the findings of Chapter Four, where 15 and 30 g (but not 0 g) HC ingestion prior to RE increased PINP concentration (as well as collagen amino acid bioavailability). The availability of the key amino acids enriched in collagen may have positively influenced collagen synthesis in through multiple mechanisms. Firstly, the increased quantity of these amino acids may have simply increased the availability of raw materials necessary to supply a RE-induced increase in collagen synthesis. Secondly, glycine, proline, and hydroxyproline (the latter of which is exclusive to collagen), may have independently stimulated collagen synthesis, as demonstrated *in vitro* (Surazynski *et al.*, 2010; Szoka *et al.*, 2017). This may have independently or synergistically with RE activated the signalling cascade associated with increased collagen synthesis in the extracellular matrix of connective tissue (i.e., Akt/ mTORCI) (Mousavizadeh *et al.*, 2020).

Regarding collagen breakdown, the decrease in plasma β-CTX concentration observed in both participants is consistent with the current available literature in young adults (Aussieker *et al.*, 2023; Lee *et al.*, 2023c) and middle-aged men in Chapter Four. The β-CTX concentration for Participant 2 (30 g HC) returned to baseline 6 h after RE, yet interestingly, β-CTX concentration continued to decrease up to 6 h post RE in

participant 1 (0 g HC), and did not return. This led to ~ 40 % lower collagen breakdown (concentration × time AUC) in Participant 1 (0 g HC) compared to Participant 2 (30 g HC), which is surprising. Henriksen *et al.* (2003) found macronutrient effect on plasma β-CTX concentration, suggesting the observed changes are unrelated to collagen or maltodextrin intake. The reason for this discrepancy is unclear and may indicate inter-individual variation in response to RE, where Participant 1 experienced a longer suppression of collagen breakdown that returned to baseline in the hours after the intervention.

In both participants, there was a small rise in serum PINP concentration 30 min after supplementation (+7.4 and + 6.3 $\mu \cdot L^{-1}$ in the 0 g HC and 30 g HC interventions, respectively) exceeded the assay sensitivity threshold of < 0.91 $\mu \cdot L^{-1.}$ Both the 0 g and 30 g HC supplements included a standardised dose of 50 mg vitamin C, an essential cofactor for collagen synthesis (Robertson & Schwartz, 1953). Vitamin C has previously been shown to upregulate collagen synthesis in dermal fibroblasts (Phillips *et al.*, 1994). Therefore, we cannot rule out the possibility that the small increase observed 30 minutes after ingestion in both participants reflects a modest effect of vitamin C, potentially arising from other collagen-rich tissues in the body

Considering the acute collagen synthesis response to 30 g HC observed in this study, resistance training (RT)-mediated increases in tendon collagen content (Kubo *et al.*, 2012) could be enhanced in the longer-term. For the purpose of this case study, we chose to provide 30 g HC during the high dose intervention, as this is the highest efficacious dose seen in Chapter Four, as well as HC dose-response studies in *young* males and long term training studies in *young* females (Lee *et al.*, 2023a; Lee *et al.*, 2023c; Lee *et al.*, 2024a). It remains unknown whether lower doses would elicit a similar response, or whether higher doses may augment collagen synthesis further

after RE, thus providing a viable direction for future research. Additionally, the long-term tendon adaptations to RT with collagen supplementation in middle-aged women are unknown. At present, only one study in middle-aged women failed to see any improvements in tendon stiffness after six months' body weight squatting without nutritional support (Kubo *et al.*, 2003). This is likely a function of insufficient overload, since in older women, tendon material properties have been shown to adapt to moderate and high intensity RE (Onambele-Pearson & Pearson, 2012; Eriksen *et al.*, 2019). Clearly, future work needs to be done to determine optimal loading and nutrition strategies to improve tendon health in middle-aged women.

***Limitations***

Strong conclusions cannot be drawn from a case study involving only two participants. This study was originally designed as a much larger randomised control trial with a crossover design, where naturally menstruating, middle-aged, resistance trained women would consume 0 or 30 g HC with RE on four occasions across two consecutive menstrual cycles, when circulating oestrogen was both high and low i.e., 0 g and 30 g HC at the onset of menses, and 0 and 30 g HC during the late follicular phase. Data collection for this study took place in early 2020 and was halted immediately due to the COVID-19 national lockdown in Ireland. This was not the only challenge that resulted in a final sample size of two participants. Firstly, although every effort was made to recruit the appropriate sample and test at the specific phases of the MC required to answer our research question, we acknowledge that self-reported menstruation status has its limitations. Additionally, since the objective of this study was to recruit pre-menopausal, yet middle-aged and not young women, which posed a unique challenge, since the identification of peri-menopausal status is difficult and nuanced (Bondarev *et al.*, 2018; Elliott-Sale *et al.*, 2021), making it difficult to recruit

large numbers of middle-aged women. When initial data collection began for this study, additional volunteers signed up and self-reported as pre-menopausal. However, over the course of tracking their menstrual cycles in anticipation of the acute intervention period, potential participants reported the onset of cycle irregularities, including cessation of menses, which may have indicated the onset of menopause, or peri-menopause. This issue meant exclusion from the study due to likely reductions in oestradiol and the inability to confidently predict menstrual cycle phases.

### *Conclusion*

Despite these limitations, the data cautiously suggest that high-intensity RE alone does not increase collagen synthesis in middle-aged, naturally menstruating, premenopausal, resistance-trained women during the late follicular phase of the menstrual cycle. Conversely, supplementation with 30 g of vitamin C-enriched HC increased collagen synthesis post-RE. These findings suggest that the timed combination of exogenous collagen ingestion and high-intensity RE may be a viable strategy to enhance connective tissue adaptations in this population.

# Chapter Six

# Collagen supplementation augments strength training-induced gains in tendon size and rate of force development in international female Master athletes

This study was presented at the 29th European Congress of Sport Science Annual Meeting as: Nulty, C. and Erskine, R.M. (2024). Hydrolysed Collagen Supplementation Augments Patellar Tendon Hypertrophy and Rate of Force Development Following Eight Weeks' Resistance Training in Female Masters Field Hockey Athletes. *Presented at the European College of Sport Science (ECSS) Congress 2024, Glasgow*.

## Prelude

Chapters Four and Five demonstrated that the systemic collagen synthesis response to a single bout of resistance exercise is optimised with the co-ingestion of 30 g hydrolysed collagen (HC) in middle-aged, resistance-trained male and female adults. These findings suggest that the combination of 30 HC ingestion might enhance connective tissue adaptations to chronic resistance training (RT) in middle-aged athletes. Accordingly, Chapter Six expands on these findings by investigating the effects of 30 g HC supplementation with eccentric focussed RT on changes in patellar tendon properties in international female Masters hockey athletes. Data collection for this study took place over 10 weeks between April and June 2023, while the international hockey squads were partaking in a pre-competition camp, preparing for the European Championships in July 2023. Since these elite athletes were primarily located at their respective clubs, based across the UK and Ireland, it was not possible for these participants to report to the University laboratory for pre- and post-testing, and only weekend training could be conducted as a group. This necessitated the development of a mobile laboratory, and the weekly transportation of supplements and RT equipment to the athletes' training camp. Despite the significant logistical challenges related to conducting this study, the study design overcame these issues and it was possible to complete an 8-week, double-blind, randomised control trial, with one week either side of the intervention utilised for data collection.

# Abstract

We investigated the effect of 8-weeks' eccentric resistance exercise (RE) with hydrolysed collagen (HC) supplementation on patellar tendon (PT) cross sectional area (CSA), vastus lateralis (VL) muscle size, maximum voluntary force (MVF) and peak rate of force development (pRFD) in international female field hockey Master athletes. Twenty-two premenopausal women (37 ± 2 years, 69 ± 8 kg; 1.68 ± 4 m) were randomly assigned to collagen (COL, n = 10) and placebo (PLA, n = 12) cohorts in a double-blind design. They completed three eccentric RE sessions per week for 8-weeks in addition to their regular hockey training. Before each RE session, participants ingested 30g HC (COL) or 30g maltodextrin (PLA), together with 500mg vitamin C. Pre- and post-intervention, we assessed MVF and pRFD during an isometric mid-thigh pull, and countermovement jump (CMJ) height. VL thickness and PT CSA were measured with ultrasonography. MVF increased from 892 ± 366 to 1,011 ± 420 N ($P = 0.020$) and VL thickness from 21 ± 3 to 22 ± 3 mm ($P = 0.015$), with no group × time interactions ($P > 0.05$), while CMJ height did not change ($P = 0.238$). PT CSA increased in both groups ($P < 0.001$) but more in COL (116 ± 12 to 121 ± 13 $mm^2$) than PLA (109 ± 22 to 111 ± 22 $mm^2$, $P = 0.014$). Similarly, pRFD increased in both groups ($P = 0.002$) but more in COL (7.9 ± 1.3 to 10.1 ± 2.4 $kN \cdot s^{-1}$) than PLA 8.2 ± 2.4 to 9.6 ± 2.9 $kN \cdot s^{-1}$, $P = 0.039$). Therefore, 8-weeks' eccentric RE with 30g HC enhanced gains in PT CSA and pRFD in elite female field hockey Master athletes, thus providing an effective strategy to improve physical performance in this under-researched population.

## 6.1 Introduction

Tendon is a metabolically active tissue (Miller *et al.*, 2005a; Smeets *et al.*, 2019) that comprises ~70% collagen (Wang, 2006) and is crucial for transferring muscle force to bone, as well as storing and releasing energy during stretch-shortening actions (Arampatzis *et al.*, 2007; Heinemeier & Kjaer, 2011). Female tendons are more compliant than those in males (Onambélé *et al.*, 2007), and older tendons exhibit greater compliance compared to younger tendons (Onambele *et al.*, 2006; Stenroth *et al.*, 2012; Quinlan *et al.*, 2018). These age and sex-differences in tendon properties are likely factors contributing to increased soft tissue injury risk in female athletes (Hewett *et al.*, 2016) and reduced athletic performance in Master athletes (Ganse & Degens, 2021).

When examining the effect of ageing on tendon properties, researchers have typically focused on extreme ends of the adult age spectrum, with only one study addressing this in non-athletic, middle-aged women, engaging in low-intensity exercise (Kubo *et al.*, 2003). As such, training and nutrition recommendations for female Master athletes often rely on extrapolation from studies in other age (and usually male) populations. Considering that sex-differences in injury rates are influenced by muscle weakness (Augustsson & Ageberg, 2017), incorporating resistance exercise (RE) into a female athlete's training programme should increase strength, thereby reducing injury risk. Furthermore, chronic RE, or resistance training (RT), leads to increased tendon cross-sectional area (CSA), stiffness, and elastic modulus (Lazarczuk *et al.*, 2022). These changes are functionally associated with a greater rate of force development (RFD) (Bojsen-Møller *et al.*, 2005; Quinlan *et al.*, 2018), and increased ultimate loading capacity (LaCroix *et al.*, 2013), which have implications for improving explosive

exercise performance, and reducing injury risk in athletes. This is important for sports such as field hockey, an Olympic stick and ball sport played across 137 international federations (IHF, 2024), which involves high-speed running and frequent explosive movements, e.g. rapid accelerations, decelerations, and changes in direction (McGuinness *et al.*, 2019). Furthermore, soft tissue injuries to the knee and lower leg were the most frequent injuries recorded in the Women's 2018 Masters (i.e. athletes aged ≥35 years) World Cup (Croteau *et al.*, 2022). Considering these demands, the ability to rapidly generate and transfer muscle force is critical for supporting explosive movements and mitigating injury risk in international level female Master athletes. However, despite the aforementioned sex differences in tendon properties and soft tissue injury risk, no study has investigated tendon properties in middle-aged female athletes, therefore the extent to which their tendons can adapt to RT remains unknown.

To facilitate training adaptation, a single bout of RE increases the fractional synthetic rate (FSR) of collagen in human tendon *in vivo* (Miller *et al.*, 2005a), while chronic RT results in increases in serum biomarkers of collagen synthesis, tendon collagen content, and tendon CSA and stiffness (Kubo *et al.*, 2012). Collagen-derived proteins/peptides, such as gelatine and hydrolysed collagen (HC), are rich in proline, glycine and hydroxyproline (Eastoe, 1955a). Due to the abundance of these amino acids in connective tissues like tendon, it is suggested that collagen ingestion may enhance connective tissue protein synthesis and aid in tissue remodelling (Shaw *et al.*, 2017), which may support training adaptation in athletes. Crucially, vitamin C is an essential co-factor during collagen synthesis (Murad *et al.*, 1981), and transport and assembly into tendon (Canty & Kadler, 2005). In this context, 30 g vitamin C-enriched collagen hydrolysate (HC) ingested before a single bout of RE augments whole body collagen synthesis more than 15 and 0 g HC in young men (Lee *et al.*, 2023c), and

although 15 g had some benefit in middle-aged men, 30 g HC was more beneficial than 15 g (Chapter Four). Furthermore, a recent case study suggests 30 g HC may mitigate the apparent negative influence of oestrogen on collagen synthesis in a naturally menstruating, resistance-trained female athlete (Lee *et al.*, 2024c). Regarding adaptation to RT with HC supplementation, Jerger and colleagues (2022; 2023) observed enhanced hypertrophy of both the Achilles and patellar tendons with HC supplementation compared to training alone in healthy young men. Similarly, the increase in tendon stiffness seen following RT in female academy (Lee *et al.*, 2023a) and professional (Lee *et al.*, 2024a) soccer players was augmented when 30 g HC was combined with RT over 10 weeks' soccer training. Finally, Lis *et al.* (2021) reported that HC supplementation helped maintain RFD following power training in field sport athletes, thus RT and HC appears to confer multiple benefits for *young* healthy and/or athletic populations, especially those performing frequent explosive movements. Despite this, no study has investigated the effects of high intensity RT on tendon size and muscle-tendon unit function during fast, explosive (high RFD) movements in pre-menopausal female Master athletes, nor whether HC could modulate these effects.

Therefore, the aim of this study was to investigate the effect of eight weeks' eccentric RT with and without HC supplementation on changes in patellar tendon properties and explosive exercise performance in elite female Master field hockey athletes. We hypothesized that RT would increase patellar tendon cross-sectional area and explosive exercise performance, and that these adaptations would be enhanced by consuming 30 g HC compared to RT alone.

## 6.2 Method

### *Experimental Design*

We recruited female Master athletes from two international field hockey squads (over 35s and over 40s) in the same training camp preparing for the 2023 European Masters Field Hockey Championships in July 2023. The over 35s were silver medallists in the World Cup 2022, and gold medallists at the European Championships in 2023; while the over 40s finished 6$^{th}$ in the World Cup 2022, and 4$^{th}$ in the European Championships 2023. Data collection began in April 2023 and was completed in June 2023. The study was a double-blind, randomized control trial where participants were pair-matched and counterbalanced into parallel groups. Prior to participation, participants were familiarized with all testing and training procedures in the first instance by video links provided with their participant information leaflets, and then during a familiarization session, which preceded the actual data collection sessions. All participants attended the national hockey training facilities (where a mobile laboratory was set up) before and after the 8-week intervention, where muscle-tendon and physical performance measures were performed. Prior to any activity, anthropometric measurements (height and body mass), as well as ultrasound measurement of vastus lateralis (VL) muscle architecture, and patellar tendon cross sectional area (CSA) were performed. This was followed by a warm-up comprising 5-min low intensity jogging, 5 high-intensity running efforts between 5 and 30 m, 3 submaximal countermovement jumps (CMJs), and 3 submaximal isometric mid-thigh pulls (IMTPs). Performance testing was carried out in the following order: CMJ, IMTP, 20 m sprint. Participants completed an 8-week lower-limb eccentric RT intervention with (COL) or without (PLA) hydrolysed collagen (HC) supplementation.

The training intervention included three sessions per week: two 'off-site' RE sessions and one supervised high-intensity flywheel squat RE session. On both pre-and post-testing occasions, participants reported to the training centre at 08:00 am, and all testing was completed by 11:00 am to avoid any potential diurnal effects on pre/post-training outcome measures. Post-training assessments were performed four days after the final supplemented training session. A schematic representation of the data collection timeline is available in Fig. 1.

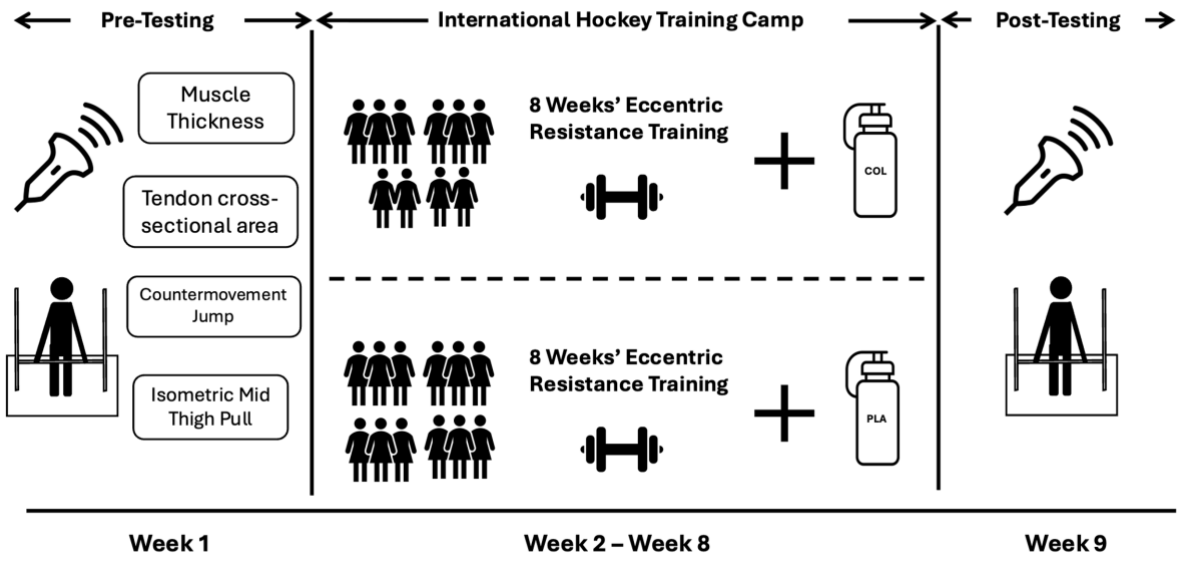


**Figure 1**. Schematic showing the timeline of data collection and resistance training plus nutritional intervention.

***Participants***

A minimal sample size was estimated prior to conducting the study with G*Power software (version 3.1.9.6, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). The estimation was performed using the effect size ($\eta_p^2 = 0.312$) from the time (pre and post 14 weeks' resistance training) × group (specific collagen peptides,

SCP vs. placebo, PLA) interaction ($p$ = 0.002) regarding the change in cross sectional area of the Achilles tendon in the study by Jerger *et al.* (2022). The results from our *a priori* power calculation deemed a minimum of 22 participants was necessary to detect an effect of COL vs. PLA (two-way analysis of variance (ANOVA); α: 0.05; power: 0.80). To account for an expected ~20% participant withdrawal (international athletes have very demanding schedules and greater likelihood of injury outside of a research study), 29 field hockey athletes from the 'over 35s' (n=19) and 'over 40s' (n=10) international playing squads volunteered and provided written informed consent prior to taking part in this study. Participants were pair-matched for height, mass, baseline MVF, menstrual cycle irregularity (n=6, see below) and oral contraceptive pill use (n=2, see below), and randomly allocated into COL and PLA based on age, height, body mass, maximal isometric strength, and self-reported hormonal status. Participants were included if they were currently selected for either the over 35s or over 40s international training squads for the 2023 season. Participants were excluded if they reported history of lower limb musculotendinous injury in the 6-months prior to commencement of the study; were vegan or vegetarian; and if they were consuming nutritional supplements that purportedly affect muscle-tendon adaptation or recovery (e.g. protein powder, vitamin C, collagen); or had a BMI > 30 $kg \cdot m^2$. Over the course of the study duration, seven participants withdrew due to non-compliance (n = 4) or injury independent of the study (n = 3; Fig. 2). Only those who were 100 % compliant with the training and nutrition intervention (n = 22; COL, n = 10; PLA, n = 12) were included in the final analysis (Fig. 2).

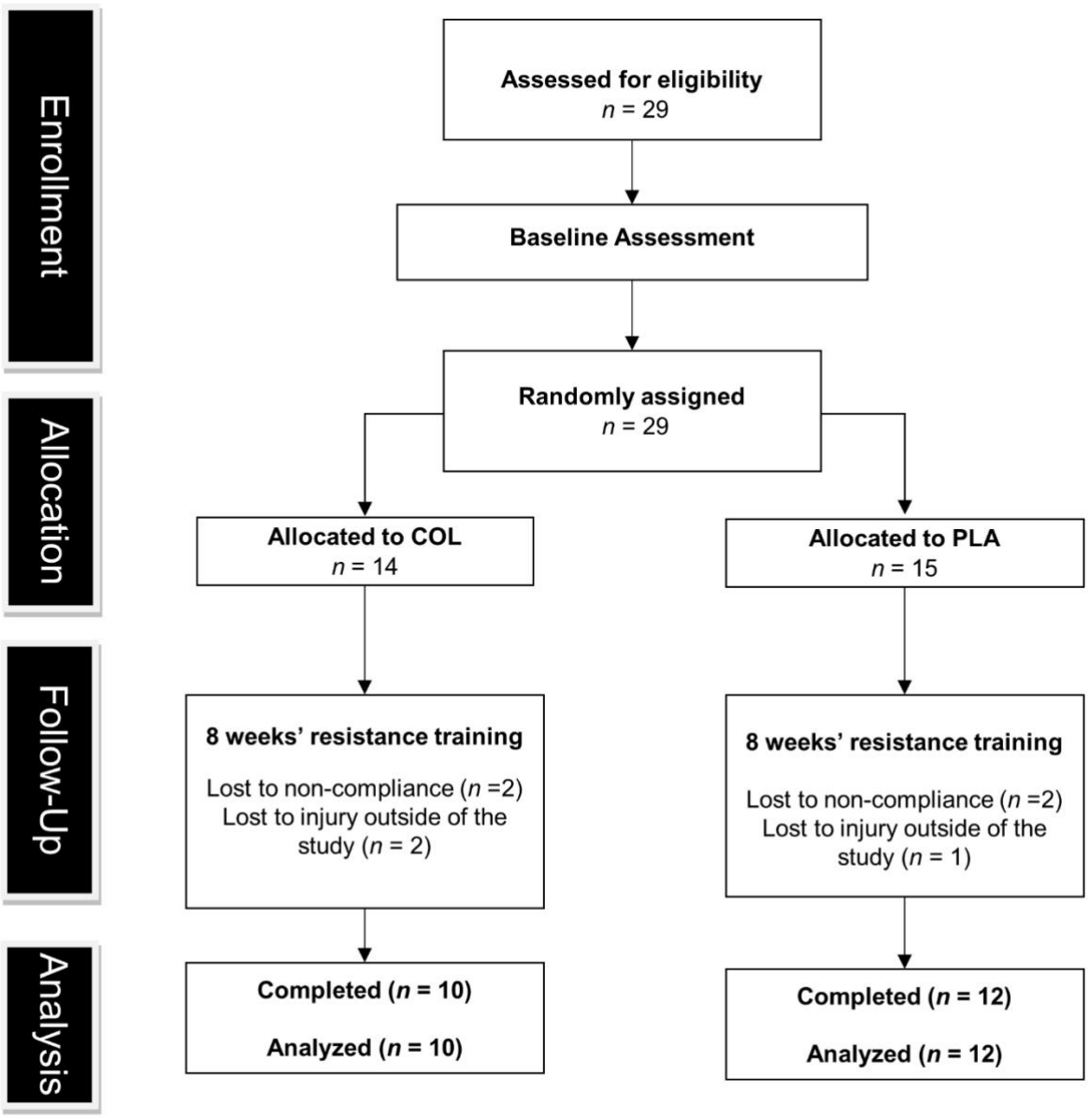


**Figure 2**. CONSORT flow diagram showing participant recruitment and intervention timeline. PLA, placebo group; COL, collagen group.

### *Identification of menstrual cycle phase and hormonal contraceptive use*

As part of the pre-screening process, participants were asked to self-report their recent menstrual cycle history via questionnaire (Appendix VII), including any current hormonal contraceptive use (delivery mode and formulation), typical menstrual cycle

length and frequency. The final analysis included naturally menstruating women with a regular cycle length of 21 to 35 days (COL, n = 7, PLA, n = 9); naturally menstruating women with at least 1 cycle length irregularity (cycle lasting < 21 or >35 days) in the past 12 months (COL, n = 3, PLA, n = 3) and combined oral contraceptive pill users (COL, n = 1, PLA, n = 1).

***Muscle architecture***

All ultrasound images were captured and analysed by the lead researcher, an operator with >10 years' experience with this technique. Portable B-Mode ultrasonography (SONOS 300L, Healcerion, Seoul, South Korea) was employed to capture sagittal images of *m. vastus lateralis* (VL) at 50% muscle length. This muscle was selected because it is the largest and most representative of the four *m. quadriceps femoris* heads (Erskine *et al.*, 2009). Participants lay supine on an elevated plinth, with all images taken from the right leg, ensuring the knee and hip were fully extended. A 38 mm wide, 10 MHz linear transducer (SONOS 300L, Healcerion, Seoul, South Korea) was used to scan four sites on the skin of the right thigh to identify the proximal and distal myotendinous junctions of the VL (to determine VL length), as well as the VL lateral and medial borders at 50% muscle length. Resting muscle architecture measurements included muscle thickness – the shortest distance between the upper and lower aponeuroses (the mean of three measurements at 25, 50 and 75% of the field of view width was used here) – and fascicle pennation angle ($\theta_p$) – the angle at which fascicles inserted into the lower aponeurosis (the mean of at least three fascicles that had clear insertions was used here) – were obtained by capturing a single image of the centre of the VL muscle belly (Fig. 3).

***Patellar tendon cross sectional area***

Participants remained supine on the elevated plinth with the right leg flexed to 90° (verified by goniometry), and a 20 kg weight plate was placed in front of the foot of the flexed leg to prevent it from sliding forward. PT length was measured first, defined as the distance from the patella apex to the insertion point of the PT into the tibial tuberosity, using the same 38 mm wide, 10 MHz linear ultrasound probe (SONOS 300L, Healcerion, Seoul, South Korea) placed over the PT in the sagittal plane. Still in the sagittal plane, three sites were identified and marked on the skin with an indelible pen (25%, 50%, and 75% tendon length – from proximal to distal ends). PT CSA was subsequently evaluated from transverse scans taken at each of the three sites, ensuring all PT borders were clearly visible, which typically required 1 to 3 scans per site. Each image was manually analysed offline by tracing the PT outline using image analysis software (Image J v1.8.0, National Institute of Health, MD, USA) (Fig. 3).

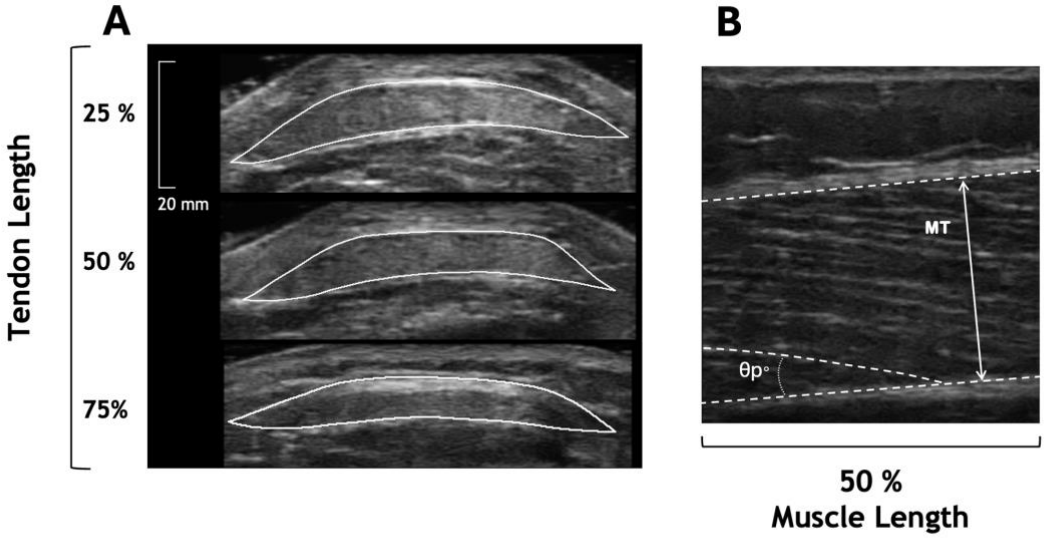


**Figure 3**. Representative image of patellar tendon cross sectional area (A) and vastus lateralis muscle architecture (i.e. muscle thickness and fascicle pennation angle, θp) (B), measured by ultrasound.

***Bilateral vertical countermovement jump***

Following the generalized warm-up described above, participants performed 3 submaximal bilateral CMJs while standing on a dual force-plates system (Force Decks FDMax, VALD Performance, Leamington Spa, UK), which was placed on a firm and flat concrete surface. For the CMJ, participants removed their shoes and stood upright with their hands on their hips throughout the jump. They were instructed to quickly squat to a self-selected depth and then immediately reverse the movement into a maximal effort jump with fully extended knees. Three maximal CMJs were performed while standing on the dual force-plates, each separated by a 1-min rest, and the CMJ with the greatest jump height pre- and post- intervention was used for subsequent analysis. The CMJ phases used for analysis were determined by the accompanying software (Force Decks Suite v.2.0.8, VALD Performance, Leamington Spa, UK). The

eccentric braking phase of the jump was defined as the time from the start of movement to the moment of minimum displacement (moment of zero velocity), while the eccentric deceleration phase began at the moment before positive vertical acceleration (maximum negative velocity) and ended at the moment of minimum displacement. The concentric phase began at the moment of minimum displacement and ended at take-off. CMJ height, modified reactive strength index ($RSI_{mod}$), and eccentric braking impulse were calculated as follows:

CMJ Height (cm) = Velocity of the Centre of Mass at the instant of Take-off and Body Mass

$RSI_{mod}$ ($m \cdot s^{-1}$) = CMJ Height ÷ by Contraction Time

Eccentric Braking Impulse (N·s) = Net Impulse (Area under the force curve over a time) during the Eccentric Braking Phase

***Maximal isometric voluntary contraction and rate of force development***

All isometric strength testing was performed on a custom rack designed to securely accommodate a portable dual force-plates system (Force Decks FDMax, VALD Performance, Leamington Spa, UK) and allow a bar to be fixed at any height that would permit an IMTP to be performed with participants of various anthropometric dimensions. All participants removed shoes and performed a specific warm-up comprising 3 submaximal efforts at 50, 60, and 70 % perceived maximal effort. For each maximal attempt, participants first performed a period of quiet standing to allow a stable force baseline to be achieved. Force-time characteristics during the IMTP were sampled at 1000 Hz and exported to a spreadsheet for offline manual analysis (Excel version 2406, Microsoft, Washington, USA). Maximal voluntary isometric force (MVF) was calculated at the peak force achieved minus the mean baseline force

during the quiet standing period, which was reported as absolute MVF, and MVF normalized to body mass (nMVF). The time of force onset was determined manually. Participants were excluded from these analyses if force-time traces during contractions were deemed unacceptable i.e. no stable weighing period, clear countermovement before initiation of movement, or peak force occurring at the end of the trial. Based on these criteria, 19 participants (COL, n = 10, PLA, n = 9) were included in the final RFD analysis. To calculate instantaneous RFD, the difference between 2 adjacent force samples were divided by the time difference between those samples (1 ms). The highest of these calculated values for any given contraction were deemed to be the pRFD (which occurred at 70 ± 2 ms after the onset of force, both pre- and post-training).

The IMTP testing was initiated by determining the necessary bar height to obtain the correct body position. This was an iterative process, in which the participant started with a bar height that allowed them to assume a body position that replicated the start of the second pull position during the 'clean': upright torso, slight flexion in the knee resulting in some dorsiflexion, shoulder girdle retracted and depressed, shoulders above or slightly behind the vertical plane of the bar, feet roughly cantered under the bar approximately hip width apart, knees underneath and in front of the bar, and thighs in contact with the bar (halfway between the iliac crest and the midpoint of the patella. The bar height was then adjusted up or down to allow the athlete to obtain the optimal knee (125–145°) and hip (140–150°) angles. These joint angles were chosen as they were shown to have the highest level of between-session reliability for pRFD when compared with several other knee- and hip- angle configurations in male collegiate athletes (intraclass correlation coefficient: 0.983, smallest detectable difference: 2.74 %) (Comfort *et al.*, 2015). Goniometry was used to ensure the same knee and hip

angles were used by each participant during pre- and post- testing. To ensure handgrip did not influence IMTP performance, all participants used heavy duty lifting straps (VersaGripps, Hancock, ME USA) for all efforts. During familiarization and again during testing, participants were taught to remove slack in the system and achieve a 'locked-in' position.

The force-time trace was visually monitored to ensure any deviations in force were < 50 N during this time. Participants were then instructed to "lock-in", and then the command was given: 2,1…GO, upon which they were cued to push the ground away as 'fast and hard as possible' to ensure their highest RFD could be achieved (Halperin *et al.*, 2016). Each contraction lasted up to 3 seconds. After a rest period of 60 s, the process was repeated, and between 2 – 4 attempts were required to achieve a useable attempt or ensure peak force was achieved (i.e. <5% greater MVF than the next highest attempt per participant).

***Nutritional supplementation***

As the participants involved in this study were all elite athletes competing at international level, all commercially available supplements had to be 'Informed Sport' certified, ensuring they were tested by Laboratory of the Government Chemist Group's anti-doping laboratory for contamination with banned substances. The dry ingredients for both the HC (COL) and maltodextrin (PLA) supplements were prepared into opaque sachets (with the participant's name clearly labelled on the outside of the sachet) by a laboratory technician independent of the study. Furthermore, the consistency and colour of these dry ingredients were indistinguishable to the naked eye, ensuring that neither the researcher nor the participant could identify the supplement or determine group assignment based on

appearance alone. The COL contained 30 g orange flavoured HC (collagen type I and III peptides, natural flavouring, acidity regulator: citric acid, anti-caking agent: silicon dioxide, 120 mg ascorbic acid, colour: beta-carotene, sweetener: sucralose; Healthspan, Guernsey, UK). The PLA was matched for taste and caloric value, and contained 32.9 g orange flavoured electrolyte-maltodextrin powder (GO-Electrolyte, Healthspan, Guernsey, UK), comprising a maltodextrin and fructose carbohydrate mix (93%), citric acid, electrolytes (2%) (sodium chloride, calcium lactate, potassium chloride, sodium citrate, magnesium citrate), natural flavouring, and aspartame. All participants were provided with a shaker bottle and given written instructions to mix the dry ingredients in 450 mL water 1 h before each RT session, consuming the mixture 30 min prior to the training session. Alongside every beverage, participants in both groups consumed 500 mg of vitamin C in a chewable tablet (Elite vitamin C, Healthspan, Guernsey, UK). Outside of the supervised training sessions, participants were instructed to maintain their habitual dietary habits for the duration of the study. Compliance with the supplementation protocol was 100% (i.e. each participant consumed all 24 supplements) – this was checked once a week by the lead researcher when players reported for their weekly in-person hockey training session.

***Resistance training intervention***

The 8-week RT intervention was designed by the researchers to be integrated into the existing hockey training program, which was prescribed by the international field hockey coaching staff. Participants performed 7 training sessions per week (3 RT sessions, 2 energy system conditioning sessions, and 2 pitch sessions), and nutritional supplementation was consumed before each of the 3 RT sessions. The entire cohort trained together at the weekend training session at the National Training Centre, while all other sessions were performed at their local field hockey facility A typical

microcycle during training camp was Monday: RT; Tuesday: energy system conditioning; Wednesday: RT followed by pitch session; Thursday: energy system conditioning; Friday: rest; Saturday: supervised RT followed by pitch session or practice game; Sunday: rest. Since these athletes were based in various clubs across the UK and Ireland, neither mid-week RT session could be supervised in-person by the researchers. Instead, instructions for these sessions were provided by PDF instruction manuals and private YouTube videos. Reminders were sent to participants by email and WhatsApp before each session and participants were encouraged to send videos and WhatsApp messages to the lead researcher, so that they could receive feedback from the researcher. These RT sessions typically comprised nine working sets of eccentric focussed exercise. To overload the lower limbs, athletes used a combination of body weight exercise e.g., reverse Nordic curls, and tempo-controlled exercise with external load e.g., Bulgarian split squat, 3 s concentric, 6 s eccentric with 10 – 20 kg external load (using a dumbbell or weight plate). The number of sets, reps and external load were standardised across all participants and progressed weekly in a linear fashion.

Supervised RT sessions comprised high-intensity squats on an isoinertial flywheel device (kBox Pro, Exxentric AB, Stockholm, Sweden). Typically, these sessions comprised 3 – 4 sets of 6 – 8 maximal effort repetitions (following 2 submaximal 'lead in' repetitions) at an inertial load of between 0.05 kg/m$^2$ and 0.075 kg/m$^2$, beginning at the lowest volume and intensity in week 1 (3 sets of 6 repetitions at 0.05 kg/m$^2$), and progressing by number of repetitions or number of sets weekly until the highest volume and intensity (4 sets of 8 repetitions at 0.075 kg/m$^2$) was reached in week 8. Compliance with the RT protocol was 100% (i.e. all participants performed all 24 RT sessions) – this was checked during the weekly in-person hockey training sessions.

The remaining of the training camp programme (i.e., energy system conditioning sessions and hockey training) were outside of the control of the researchers and exclusively under control of the international coaching staff.

***Statistical Analysis***

All data were analysed using the statistical software package for social sciences (SPSS v. 29, IBM, Armonk NY, USA), reported as mean ± standard deviation with significance accepted at $P < 0.05$. Independent t-tests were performed to assess whether there were any group differences between COL and PLA on any variable measured at baseline. Effects of the intervention on all variables were assessed for main effects of time, group, and time × group interaction effects by two-way analysis of variance (ANOVA). The only exception to this was PT CSA, which also included an effect of location along the tendon length, thus a three-way ANOVA (time × group × location) was used. Where main effects or interaction effects were observed, post-hoc paired t-tests were used for pairwise comparisons (pre- to post-intervention) within groups, and independent t-tests were used to compare the percentage change scores between groups. Partial eta squared, $\eta_p^2$ (for ANOVA effects), and Cohen's $d$ (t-tests) were reported as estimates of effect size for each respective statistical model. The thresholds of $\eta_p^2$ and Cohen's $d$ are defined as small ($\eta_p^2 = 0.01$ and $d = 0.20$), medium ($\eta_p^2 = 0.06$, $d = 0.50$ and) and large ($\eta_p^2 = 0.14$, $d = 0.80$) (Cohen, 2013).

## 6.3 Results

***Group characteristics***

There were no differences in body mass, height, or age between groups at baseline ($p > 0.05$). There were also no changes in body mass following the intervention (time: $F_{1,20} = 2.768$, $p = 0.113$; time × group: $F_{1,20} = 0.267$, $p = 0.611$).

***Muscle architecture***

There were no differences in muscle architecture between groups at baseline ($p > 0.05$). Muscle architecture and strength changes are presented in Table 1. VL MT increased from pre- to post- intervention (21.2 ± 3.5 to 21.8 ± 3.4 mm, + 7 %, time: $F_{1,20} = 7.126$, $p = .015$, $\eta_p^2 = 0.263$) but there was no time × group interaction ($F_{1,20} = 2.946$, $p = .102$). Similarly, VL $\theta_p$ increased following the intervention (13.2 ± 2.0 to 14.4 ± 2.5°, + 20 %, time: $F_{1,20} = 4.864$, $p = .039$) with no time × group interaction ($F_{1,20} = 0.599$, $p = 0.448$).

***Maximum isometric strength***

There was no difference in MVF between groups at baseline ($p > 0.05$). Both absolute and normalized (to body mass) MVF increased ($F_{1,20} = 4.636$, $p = 0.044$, $\eta_p^2 = 0.188$; $F_{1,20} = 7.013$, $p = 0.016$, $\eta_p^2 = 0.280$), with no time × group interaction on either measure ($F_{1,20} = 0.022$, $p = 0.884$, $F_{1,20} = 0.005$, $p = 0.944$) (Table 1).

**Table 1.** Muscle, strength and vertical power adaptations to 8-weeks' eccentric RT with and without collagen supplementation. Data are mean ± SD

| Variable | COL Pre | COL Post | Delta % | PLA Pre | PLA Post | Delta % | p-value, Time | p-value, Group × Time |
|---|---|---|---|---|---|---|---|---|
| Iso MVC (N) | 850 ± 255 | 945 ± 314 | 13 ± 22 | 937 ± 497 | 1014 ± 561 | 15 ± 37 | 0.044 | 0.884 |
| Iso MVC (N/kg) | 12.7 ± 2.7 | 14.0 ± 3.9 | 10 ± 23 | 13.4 ± 4.9 | 15.9 ± 6.2 | 17 ± 36 | 0.016 | 0.431 |
| VL Muscle Thickness (mm) | 22.4 ± 2.9 | 22.6 ± 3.0 | 1 ± 4 | 20.2 ± 3.7 | 21.3 ± 3.7 | 6 ± 7 | 0.022 | 0.113 |
| VL Fascicle $\theta_p$ (°) | 13.4 ± 1.6 | 13.9 ± 2.0 | 10 ± 15 | 12.8 ± 2.3 | 14.5 ± 3.0 | 7 ± 19 | 0.016 | 0.184 |
| Jump Height (cm) | 21.7 ± 4.6 | 23.8 ± 6.9 | 7 ± 28 | 23.7 ± 3.7 | 23.8 ± 3.4 | 4 ± 23 | 0.238 | 0.280 |
| RSI-modified (Imp-Mom) (m·s-1) | 0.24 ± 0.05 | 0.25 ± 0.08 | 0.0 ± 0.1 | 0.25 ± 0.06 | 0.25 ± 0.06 | 0.0 ± 0.1 | 0.484 | 0.691 |
| Eccentric Braking Impulse (N·s) | 28.0 ± 11.5 | 36.1 ± 11.2 | 50 ± 86 | 37.3 ± 16.4 | 33.9 ± 15.7 | 0 ± 41 | 0.365 | 0.036 |

*Iso, Isometric; MVC, maximal voluntary contraction; VL, vastus lateralis; $\theta_p$ fascicle pennation angle; RSI, reactive strength index*

***Rate of force development***

There was no difference in pRFD between groups at baseline ($p > 0.05$). There was an increase in pRFD following the intervention ($F_{1,17} = 14.26$, $p = 0.002$, $\eta_p^2 = 0.456$) and a time × group interaction ($F_{1,17} = 5.02$, $p = 0.039$, $\eta_p^2 = 0.228$). The 27.3 ± 19.2 % increase in COL was greater than the 8.0 ± 19.6 % increase in PLA ($t_{1,18} = 2.16$, $p = 0.045$, $d = 0.994$) (Fig. 4). Time to pRFD remained unchanged (time: $F_{1,17} = 1.100$, $p = 0.309$, time × group: $F_{1,17} = 0.145$, $p = 0.708$).

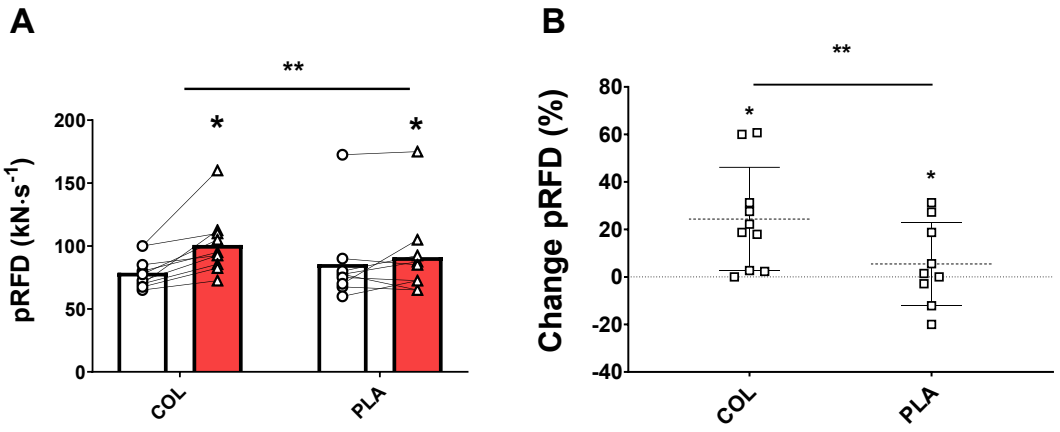


**Figure 4. A**. Peak rate of force development (pRFD) before (circles, white bar) and after (triangles, red bar) 8-weeks' eccentric RT, with (COL) and without (PLA) collagen supplementation, and **B.** Mean ± SD percentage change in pRFD following 8-weeks' eccentric RT. * Post than pre ($p < 0.05$); ** time × group interaction ($p = 0.028$); # Independent t-test, COL greater than PLA ($p = 0.021$).

***Patellar tendon morphology***

Mean PT CSA did not differ between groups at baseline (COL: 115.7 ± 12.3 $mm^2$ vs. PLA: 109.1 ± 22.3 $mm^2$, $t_{20} = 0.841$, $p = .410$), and all pre- and post-training data are displayed in Fig. 5. Following 8 weeks' RT there was an effect of time ($F_{1,20} = 36.482$, $p < .001$, $\eta_p^2 = 0.646$), and a time × group interaction ($F_{1,20} = 7.302$, $p = .014$, $\eta_p^2 =$

0.267), where mean PT CSA increased 4.6 ± 2.7 % ($t_9$ = 5.361, p < 0.001, $d$ = 1.7) in COL and 2.0 ± 2.8 % in PLA ($t_{11}$ = 2.732, p = .020, $d$ = 0.789), and the increase in COL was greater than the increase in PLA ($t_{20}$ = 2.18, p = 0.021, $d$ = 0.935).

Regarding regional PT CSA, there was no time × location × group three-way interaction ($F_{2, 40}$ = .172, p = .842) but there was a time × location interaction ($F_{1, 20}$ = .7.326, p = .014). At 25% tendon length, there was a time effect ($F_{1, 20}$ = 13.627, p = .001, $\eta_p^2$ = 0.405) and a time × group interaction ($F_{1, 20}$ = 6.034, p = .023, $\eta_p^2$ = 0.232). COL showed an increase from 120.8 ± 15.7 mm² to 125.3 ± 15.2 mm² ($t_9$ = -3.2, p = .010, $d$ = 1.03), while PLA showed no change from 112.7 ± 23.4 mm² to 113.6 ± 23.9 mm² ($t_{11}$ = -1.3, p = .210, $d$ = 0.385). At 50% tendon length, there was no effect of time ($F_{1, 20}$ = 3.931, p = .061) and no time × group interaction ($F_{1, 20}$ = 0.838, p = .371). At 75% tendon length, there was a main effect of time (F1, 20 = 22.346, p < .001, $\eta_p^2$ = 0.528) but no time × group interaction ($F_{1, 20}$ = 2.669, p = .118). COL showed an increase from 116.4 ± 13.1 mm² to 124.2 ± 12.7 mm² ($t_9$ = -4.1, p = .003, $d$ = 1.30), and PLA also showed an increase from 112.3 ± 21.6 mm² to 116.1 ± 22.3 mm² ($t_{11}$ = -2.4, p = .035, $d$ = 0.692).

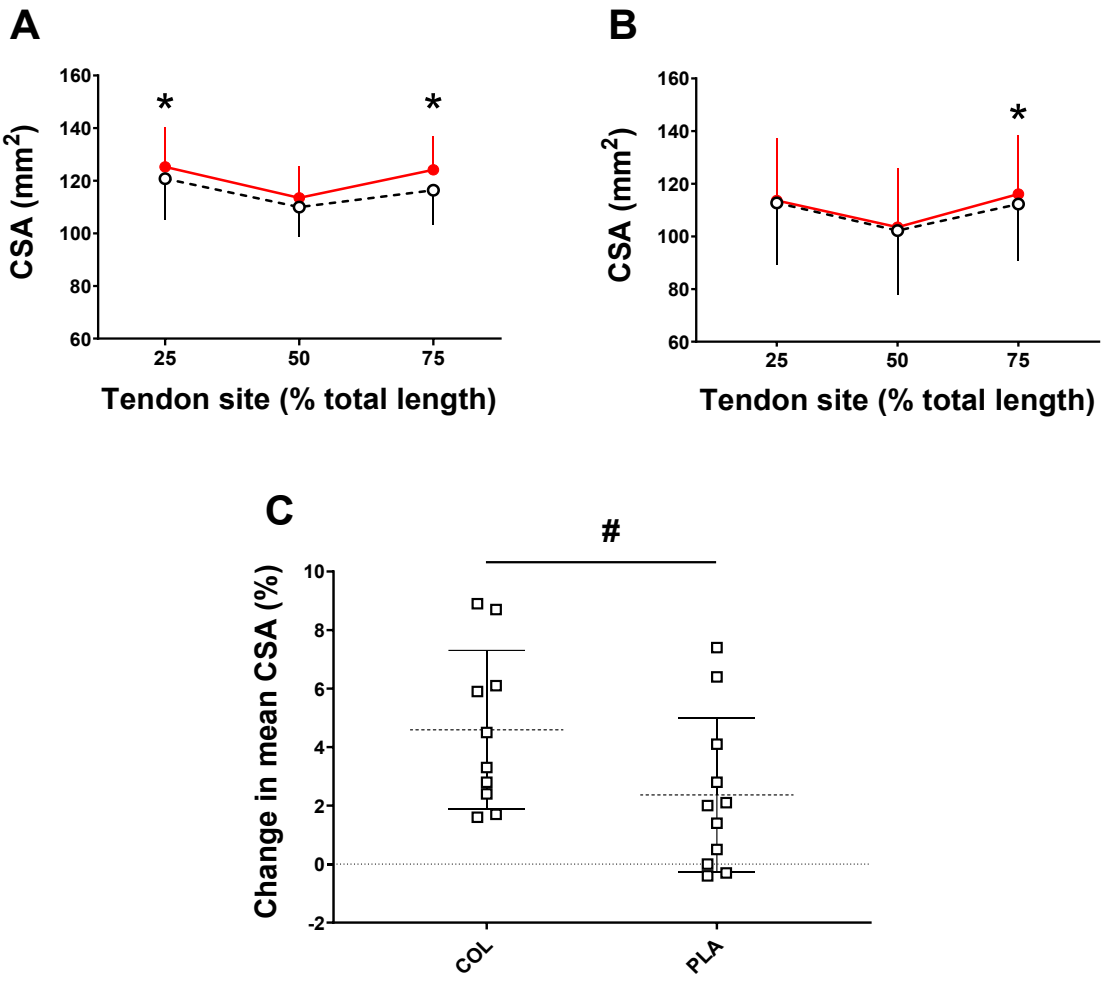


**Figure 5.** Patellar tendon cross sectional area (CSA) at 25, 50, and 75 % tendon length before (white circles) and after (red circles) 8-weeks' RT in COL (**A**) and PLA (**B**), and. **C**. Mean ± SD percentage change in PT CSA. * Post greater than pre ($P < 0.05$); # Independent t-test, COL greater than PLA ($p = 0.04$).

### *Vertical power performance*

CMJ height and $RSI_{mod}$ remained unchanged following the intervention ($F_{1,20} = 1.478$, $p = 0.238$; $F_{1,20} = 0.508$, $p = 0.484$, respectively) and there were no group × interactions ($F_{1,20} = 1.478$, $p = 0.280$; $F_{1,20} = 1.478$, $p = 0.691$, respectively). There was no effect of

time on eccentric braking impulse ($F_{1,20}$ = .861, p = 0.365, $\eta_p^2$ = 0.043) but there was a group × time interaction ($F_{1,20}$ = 5.080, p = 0.036, $\eta_p^2$ = 0.211). Post-hoc pairwise comparisons showed that there was an increase in eccentric braking impulse in COL (28.0 ± 11.5 to 36.1 ± 11.2 N·s, p = 0.043, *d* = 0.650) but not in PLA (36.1 ± 11.2 to 33.9 ± 15.7 N·s, p = 0.304, *d* = 0.311).

## 6.4 Discussion

The purpose of this study was to determine whether HC supplementation augmented changes in PT morphology and explosive exercise performance in elite pre-menopausal female field hockey Master athletes. Ingestion of 30 g HC alongside 500 mg vitamin C three times per week before eccentric RT resulted in greater increases in PT CSA and pRFD during isometric contraction compared to RT alone.

Our findings that maximal strength and muscle size increased by 14 and 7%, respectively, following eight weeks' eccentric RT are in line with previous RT studies (Erskine *et al.*, 2010; Erskine *et al.*, 2014; Folland *et al.*, 2014). Moreover, the lack of any between group differences in the magnitude of these changes was also unsurprising. Several studies have now shown that collagen supplements are unlikely to confer greater increases in muscle strength (Kirmse *et al.*, 2019b; Jacinto *et al.*, 2022a; Jerger *et al.*, 2022; Jerger *et al.*, 2023; Lee *et al.*, 2023a; Lee *et al.*, 2024a). This is to be expected given that skeletal muscle comprises only a small amount of collagen (Gillies & Lieber, 2011), and 30 g HC has been shown to be ineffective for enhancing RE-induced myofibrillar and skeletal muscle connective tissue FSR (Oikawa *et al.*, 2020; Aussieker *et al.*, 2023).

The increases in PT CSA observed in female Master athletes in our study (PLA: + 2 ± 3%, COL: + 5 ± 3%) are slightly lower than the 6 ± 2% increase in tendon volume

reported following 8 weeks' RT in young women with no nutritional supplementation (McMahon *et al.*, 2018). However, we observed that the most pronounced changes occurred in the distal portion of the tendon (COL: + 5.3%, PLA: +3.5 %) which aligns with previous work, demonstrating that the majority of tendon hypertrophy occurs at the osteotendinous junction (Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009). Considering the baseline CSA was much larger in our study of international athletes [likely due to habitual loading patterns and athletic backgrounds (Wiesinger *et al.*, 2016)], they may have experienced diminishing returns compared with young, previously untrained women. Alternatively, there may be some effect of increasing age, as a small number of studies have observed no increase in tendon stiffness (CSA not reported) in middle-aged women performing bodyweight training (Kubo *et al.*, 2003) and no increase in PT CSA of older women (> 65 years) performing chronic RT (Reeves *et al.*, 2003a; Onambele-Pearson & Pearson, 2012; Grosset *et al.*, 2014). However, these studies may not have met the RT intensity threshold required for tendon hypertrophy (Bohm *et al.*, 2015), or may have had slight variations in methodology, such as measuring different sites along tendon length. In contrast, one study has shown that female Achilles tendon CSA increased by 6 % in older women after 14-weeks' high strain exercise on a dynamometer, although no further increases were observed after 1.5 years' RT (Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009; Epro *et al.*, 2017).Nonetheless, we have demonstrated for the first time that, in middle-aged, pre-menopausal female athletes, the PT remains mechanosensitive to high intensity RT.

The roughly doubling of tendon hypertrophy seen in COL compared with PLA suggests a synergistic effect of high intensity RT and collagen supplementation similar to that reported in previously untrained young healthy men (Jerger *et al.*, 2023),

despite that study reporting larger relative changes (+5 % PLA, + 11% COL), which again may be linked to the difference in age and/or habitual loading patterns of the tendon between studies. We chose to provide 30 g HC (with vitamin C) prior to each RE session based on the findings of Chapter Three, where 30 g vitamin C-enriched HC increased serum procollagen type I N-terminal propeptide (PINP, a marker of whole-body collagen synthesis) in middle-aged men to a greater extent than 15 g HC. This protocol has also been shown to increase serum PINP concentration after RE in both a 36 year old resistance-trained female athlete (Lee *et al.*, 2024c) and a 42 year old resistance-trained female athlete (Chapter Four). In our study, the provision of exogenous collagen likely increased PT hypertrophy by augmenting the RT collagen synthesis response over time due to the greater abundance of key amino acids providing more raw materials for tendon collagen formation. Alternatively (or in addition), the greater bioavailability of collagen amino acids may have independently stimulated collagen synthesis through various signaling pathways, as demonstrated using *in vitro* models (Surazynski *et al.*, 2010; Schunck & Oesser, 2013; Szoka *et al.*, 2017).

Furthermore, we have shown for the first time that collagen supplementation can enhance RT improvements in pRFD. RFD is a complex and multifactorial trait, influenced by neuromuscular factors such as maximal strength, muscle size, fiber type and myosin heavy chain isoform composition, and motor unit recruitment and discharge rate (Häkkinen & Komi, 1986; Harridge *et al.*, 1996; Folland *et al.*, 2014; Maffiuletti *et al.*, 2016). Therefore, it is likely that some combination of these factors led to improvements in RFD after 8-weeks RT observed in our study (Aagaard *et al.*, 2002), although greater tendon stiffness is also associated with higher RFD (Bojsen-Møller *et al.*, 2005; Quinlan *et al.*, 2018). There are two mechanisms by which

mechanical loading induces changes in tendon stiffness: the first being an increase in collagen fibril density and cross-linking (Heinemeier & Kjaer, 2011; Couppé *et al.*, 2021), and the other being and increase in the CSA of the tendon (Arampatzis *et al.*, 2007; Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009). It is therefore logical that the greater increases in PT CSA in COL observed in our study likely led to a greater increase in tendon stiffness, which in turn, contributed to the greater improvements in pRFD compared with PLA.

Interestingly, we also observed improvements in eccentric impulse during CMJ in COL but not in PLA. The greater improvements in pRFD and eccentric braking impulse in COL would be expected to positively influence dynamic hockey performance. A higher isometric pRFD during a multi-joint assessment like IMTP is related to faster sprint times and greater performance in field-based agility tests (Wang *et al.*, 2016). Indeed, in a similar training protocol to our study, 10 weeks' eccentric overload training using flywheel squats increased braking impulse and improved change of direction performance in male football players (de Hoyo *et al.*, 2016). Furthermore, Lis *et al.* (2021) found that short term power training combined with HC improved eccentric braking impulse and maintained RFD compared to training alone, which supports our findings. These improvements are crucial for field hockey players, as a greater eccentric braking impulse should facilitate greater storage and release of elastic energy (Spiteri *et al.*, 2013). Furthermore, eccentric strength plays a fundamental role in change of direction performance (Brughelli *et al.*, 2008), which is key in field hockey where players are required to effectively mark opponents, evade defenders, and achieve positional advantages (Spencer *et al.*, 2004).

Despite the increase in pRFD and eccentric braking impulse, there was no corresponding improvement in CMJ height following eight weeks' eccentric RT,

which is in contrast to previous work (Raya-González *et al.*, 2022). This outcome may be attributed to the current study cohort's low $RSI_{mod}$, indicating a poor ability to transfer force generated during eccentric loading to concentric propulsion in the vertical plane. Moreover, the high variability between participants likely masked any potential effects of the intervention, possibly due to the lack of necessity for vertical plane movements in field hockey, leading to unfamiliarity with the task. Nonetheless, it could be expected that the greater improvements in eccentric braking impulse and pRFD in COL could potentially reduce impact forces during explosive movements like rapid change of direction.

Furthermore, tendon hypertrophy following RT is usually associated with augmented tendon stiffness (Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009). As a stiffer tendon can tolerate greater stress (LaCroix *et al.*, 2013) and transmit muscle force more efficiently (Bojsen-Møller *et al.*, 2005), the practical implications of our findings may not be limited to improved physical performance but may also help mitigate tendon injury risk. Overloading also has beneficial effects on other connective tissues, such as ligament (Kharaz *et al.*, 2021). Thus, given the similar collagen composition of tendon and ligament, it is feasible that the benefits of RT supplemented with HC might not be limited to tendon, and may even help reduce ligament injury risk, although this currently remains speculative.

***Limitations***

We used ultrasound to capture images of tendon CSA. Typically, magnetic resonance imaging (MRI) is considered the 'gold standard', but this was not possible in a mobile laboratory, which was required to assess the elite athletes in this study. Despite this, the agreement between ultrasound and MRI measurement of PT CSA is very high ($r^2$

= 0.99, p < 0.0001) (Quinlan *et al.*, 2021), especially in experienced operators (Stenroth *et al.*, 2019) like the researcher in our study who had >10 years' experience at the time of data collection. Furthermore, the reproducibility of ultrasound tendon CSA measurements in our laboratory is very high, with CVs and ICCs ranging from 1-2% and 0.984-0.997, respectively (Murtagh *et al.*, 2018; Lee *et al.*, 2023a). In addition, a recent case study has shown that whole body collagen synthesis following RE is affected by serum oestrogen concentration (Lee *et al.*, 2024c), so we acknowledge that not controlling for menstrual cycle phase during the pre- and post-training assessments may have had some influence on our results. We also acknowledge that the inclusion of six participants with at least one cycle length irregularity in the past 12 months, and two oral contraceptive pill users, may have impacted our data. However, patellar tendon properties (the main target of our RT and nutritional intervention) are unaffected by menstrual cycle phase (Hansen *et al.*, 2009a) and oral contraceptive use (Hansen *et al.*, 2013b). Notwithstanding, testing all participants at the same menstrual cycle phase pre- and post-training would not have been possible due to the international athletes' limited availability. Furthermore, to limit any potential influence of menstrual cycle irregularity or oral contraceptive pill use on between group differences, those participants with at least one irregular menstrual cycle in the past 12 months (n = 6) and oral contraceptive pill users (n = 2) were allocated equally between groups.

***Conclusion***

In conclusion, eight weeks' eccentric RT supplemented with 30 g hydrolysed collagen three times per week resulted in greater gains in patellar tendon size and isometric pRFD compared to training alone in elite female Masters field hockey athletes. These

findings have positive implications for pre-menopausal female Master athletes seeking to improve explosive exercise performance.

# Chapter Seven

# Hydrolysed collagen supplementation enhances patellar tendon adaptations to 12-weeks' resistance training in middle-aged men

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## Prelude

Chapter Six demonstrated that 30 g hydrolysed collagen (HC) ingestion with 8-weeks' eccentric focussed RT augments increases in tendon size and rate of force development in female Master athletes. Considering the effects of RT on tendon properties are purported to differ between sexes, and following the positive effects observed in middle-aged women in Chapter Six, this final experimental chapter investigates the effects of chronic RT with collagen supplementation in middle-aged men. Since this study was conducted on participants who were local to the University, it was possible to complete a longer intervention i.e., 30 g HC with 12-weeks' RT, and include laboratory-based measures of tendon stiffness that were not possible using the mobile laboratory in Chapter Six. Data collection for this study began in July 2021, as soon as COVID-19 national lock-down restrictions in Ireland were eased. Since gyms and leisure facilities had been closed since March 2020, it was decided that recruitment for this study should include middle-aged men, who are recreationally active, but naïve to RT. For the duration of this study, RT was conducted in 'pods' of no more than three participants per training session, with equipment separated for the purpose of social distancing. Data collection for this study was completed in December 2022.

# Abstract

Resistance exercise (RE) with hydrolysed collagen (HC) supplementation increases collagen synthesis in young and middle-aged populations, and further enhances tendon adaptations to chronic RE in young athletes. However, it is unknown if middle-aged tendon can benefit from chronic RE with HC supplementation. We investigated the effects of 12-weeks' RE combined with HC supplementation on changes in patellar tendon (PT) properties in *middle-aged men*. In a double-blind design, 20 recreationally active men (age, 47±5 years) were randomly assigned to placebo (PLA, *n*=11) or collagen (COL, *n*=9) groups. Both cohorts completed progressive lower-limb RE twice weekly for 12-weeks, and were supplemented post-RE with COL (30g HC, 50mg vitamin C) or PLA (30.5g maltodextrin, 50mg vitamin C). The following were assessed before and after the 12-week intervention: barbell back squat 10-repetition maximum (10-RM); vastus lateralis (VL) muscle thickness and PT cross-sectional area (CSA at 25, 50 and 75% tendon length) using ultrasonography; isometric knee extension maximum voluntary torque (MVT) and peak rate of torque development (pRTD), PT stiffness (*k*), and Young's modulus ($\mathcal{E}$) using ultrasonography and isokinetic dynamometry. MVT, pRTD, and 10-RM increased ($P$ <0.05) with no group×time interaction ($P$>0.05), whereas VL muscle thickness remained unchanged ($P$= 0.308). Mean PT CSA increased more in COL (+6.8 ± 5.4 mm²) than PLA (+1.2 ± 2.1mm², group × time $P$ = 0.027). Similarly, *k* and $\mathcal{E}$ increased more in COL (*k*, +661 ± 331 N/mm; $\mathcal{E}$, +0.21 ± 0.13 GPa) than PLA (*k*, +247 ± 305 N/mm, group×time $P$ = 0.009; $\mathcal{E}$, +0.09 ± 0.13 GPa, group × time, $P$ = 0.018). In conclusion, 12-weeks' RE with 30g HC supplementation augmented gains in PT CSA, stiffness, and Young's modulus in *middle-aged* men.

# 7.1 Introduction

Tendon is a fibrous connective tissue that attaches muscle to bone. The dry mass of healthy human tendon comprises ~60 - 80% type I collagen fibres, which are arranged longitudinally in bundles (Gelse *et al.*, 2003; Wang, 2006). Functionally, tendons are responsible for force transmission from muscle to bone, which contributes to various biomechanical tasks including locomotion, and dynamic strength and power performance (Kubo *et al.*, 2007; Maffiuletti *et al.*, 2016). However, there is an age-related decline in force and power production that begins in middle-age (Pearson *et al.*, 2002; Kostka, 2005; König *et al.*, 2018b). Although human tendon cross-sectional area does not appear to be affected by age (Carroll *et al.*, 2008; Couppe *et al.*, 2009), middle-aged and older tendon has been shown to have reduced collagen content (Couppe *et al.*, 2009) as well as reduced stiffness and elastic modulus *in vivo* (Kubo *et al.*, 2003; Onambele-Pearson & Pearson, 2012; Stenroth *et al.*, 2012; Quinlan *et al.*, 2018), which has been associated with a lower rate of torque development (RTD) in older men (Quinlan *et al.*, 2017). These ageing-induced adaptations may have negative implications for injury susceptibility (e.g. by reducing the ability to prevent a fall), and for athletic performance.

High intensity resistance exercise (RE) is an effective stimulus to remodel the morphological (size) and mechanical (stiffness) properties of human tendons (Bohm *et al.*, 2015; Lazarczuk *et al.*, 2022), which has the potential to attenuate declines in performance associated with middle-age. These adaptations are underpinned by an increase in collagen content and cross-linking of collagen fibrils within the tendon in response to repeated, RE-mediated collagen synthesis (Miller *et al.*, 2005a; Miller *et al.*, 2007; Couppe *et al.*, 2009; Kjær *et al.*, 2009; Lee *et al.*, 2023c). In older adults

aged > 65 years, the patellar tendon is capable of increasing its stiffness and Young's modulus following chronic RE (Reeves *et al.*, 2003a), although these adaptations likely occur at a slower rate compared with *young* men (Quinlan *et al.*, 2021). It is not yet known, however, how tendon adapts to chronic RE in middle-aged men, an under-researched population in the literature.

To further enhance tendon adaptations to chronic RE, recent studies have shown that supplementing RE with hydrolysed collagen (HC) ingestion can increase tendon CSA (Jerger *et al.*, 2022; Jerger *et al.*, 2023) and tendon stiffness and Young's modulus (Lee *et al.*, 2023a; Lee *et al.*, 2024a). This is probably a consequence of both RE-induced collagen synthesis and an increased bioavailability of collagen amino acids, which may stimulate collagen synthesis independently of RE (Lee *et al.*, 2023c). However, all these studies were performed in young participants and older tendon may adapt differently to chronic RE with HC ingestion. We have recently shown that HC ingestion prior to RE leads to an increase in collagen synthesis in a dose-response manner in middle-aged resistance-trained men (Chapter Four) . In that study, 30 g HC ingestion led to a greater whole body collagen synthesis response than 15 g ingestion, which in turn was greater than the 0 g HC intervention. Interestingly, collagen synthesis did not change throughout the 0 g HC intervention, suggesting reduced sensitivity regarding a collagen synthesis response to RE in middle-aged men. The finding that 15 g HC recovered the collagen synthesis response to RE, and that 30 g augmented this response further, highlights the importance of supplementing chronic RE with HC to potentially augment tendon adaptations in middle-aged individuals. However, to date, no study has investigated whether HC supplementation can affect changes in tendon properties following chronic RE in this population.

Therefore, we aimed to investigate the effects of 12-weeks' progressive lower-limb resistance exercise combined with 30 g HC supplementation on patellar tendon stiffness, Young's modulus, and cross-sectional area in recreationally active (but naïve to resistance training), middle-aged men. Secondly, we aimed to describe the effects of this intervention on changes in lower body strength and power, assessed by knee-extensor maximum voluntary torque (MVT), leg press strength, countermovement jump (CMJ) height and broad jump (BJ) distance. We hypothesized that middle-aged men supplemented with vitamin C-enriched hydrolysed collagen would demonstrate augmented adaptations in patellar tendon properties following 12 weeks' lower-limb RT compared to RT alone, which would translate into improved physical performance.

# 7.2 Methods

### *Participants*

*A priori sample size estimation:* A minimal sample size was estimated prior to conducting the study with G*Power software (version 3.1.9.6, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). This estimation was based on the results from the only study published prior to the start of the present study that examined the effect of nutritional supplementation with resistance training (RT) on patellar tendon cross-sectional area (CSA) (Farup *et al.*, 2014), i.e. the main outcome measure in the present study. These authors reported an increase in tendon CSA of 14.9 ± 3.1% and 8.1 ± 3.2% in the treatment and placebo groups, respectively (Cohen, 2013). Thus, we used a large effect size ($f$ = 0.75) to calculate the minimum sample size required for the present study, which indicated that at least 18 participants were

required to detect an effect of COL vs. PLA (two-way analysis of variance (ANOVA); α: 0.05; power: 0.80). To account for an expected participant withdrawal of ~20%, and to ensure the study remained statistically powered, 24 healthy middle-aged men, who were physically active or involved in recreational sport (>120 min moderate activity and/or sport training per week) but naïve to lower limb resistance exercise, provided written informed consent to participate in this study. Volunteers were aged between 40 and 60 years, with no history of lower limb injury in the last 12 months. They were also required to be non-smokers and could not be vegan or vegetarian as the HC supplement was derived from bovine connective tissue. Participants were recruited by word of mouth as well as flyers posted at local sports clubs, on the University campus, and on the University social media platforms. Four participants were unable to adhere to the training intervention due to personal commitments and were removed from the study. Therefore, 20 participants completed the study (PLA: n = 11, age, 46 ± 5 years, height, 181 ± 6 cm, body mass, 84 ± 12 kg; COL: n = 9, age, 48 ± 5 years, height, 174 ± 6 cm, body mass, 78 ± 12 kg). The study was registered at https://clinicaltrials.gov/ (identifier: NCT06402890), was approved by the Ethics in Research Committee at South East Technological University (Approval No. 300/2021) and complied with the Declaration of Helsinki.

***Experimental Design***

This study was a double-blind, randomized control design with parallel groups (Figure 1). Data collection began in July 2021 and was completed in December 2021. Following familiarisation, participants were pair-matched for age, body mass, isometric knee extension strength, barbell back squat 10 repetition maximum (10-RM), assigned to one of two groups (COL or PLA). Participants attended an initial visit to the laboratory where they were screened, provided with information on how to

complete a 3-day food diary, and familiarized with all the baseline measurements and procedures. As the participants were naïve to resistance exercise, each participant was provided with instruction and coaching on back-squat technique from an experienced sport and exercise scientist. Upon returning to the laboratory and before engaging in any physical activity, muscle architecture and patellar tendon cross-sectional images were obtained. Participants completed a general warm-up, comprising 5 min low-intensity jogging and 5 min dynamic stretching before baseline measurements, which were conducted in the following order: (1) muscle architecture, (2) patellar tendon CSA, (3) bilateral vertical countermovement jump (CMJ) and broad jump, (4) tendon pre-conditioning with 10 progressive isokinetic knee extensions/flexions at $60°\cdot s^{-1}$, (5) maximal isometric knee extension and flexion assessments, (6) ramped isometric knee extension (patellar tendon mechanical properties), and (7) 10-RM back squat. Participants then completed a 12-week progressive lower-limb RT program, with two supervised RT sessions performed per week. For measurements 1 and 2, all testing was conducted on the participant's right leg. Supervised training sessions took place twice a week and involved barbell back squats, dumbbell Romanian deadlifts, trap-bar deadlifts, and dumbbell goblet squats. Training load was adjusted and progressed weekly, based on performance in the same session the previous week. Immediately after each training session was completed, participants consumed their assigned supplement (COL or PLA) under the supervision of the researchers. Participants returned to the laboratory 72 h after completion of their final training session to repeat all baseline assessments.

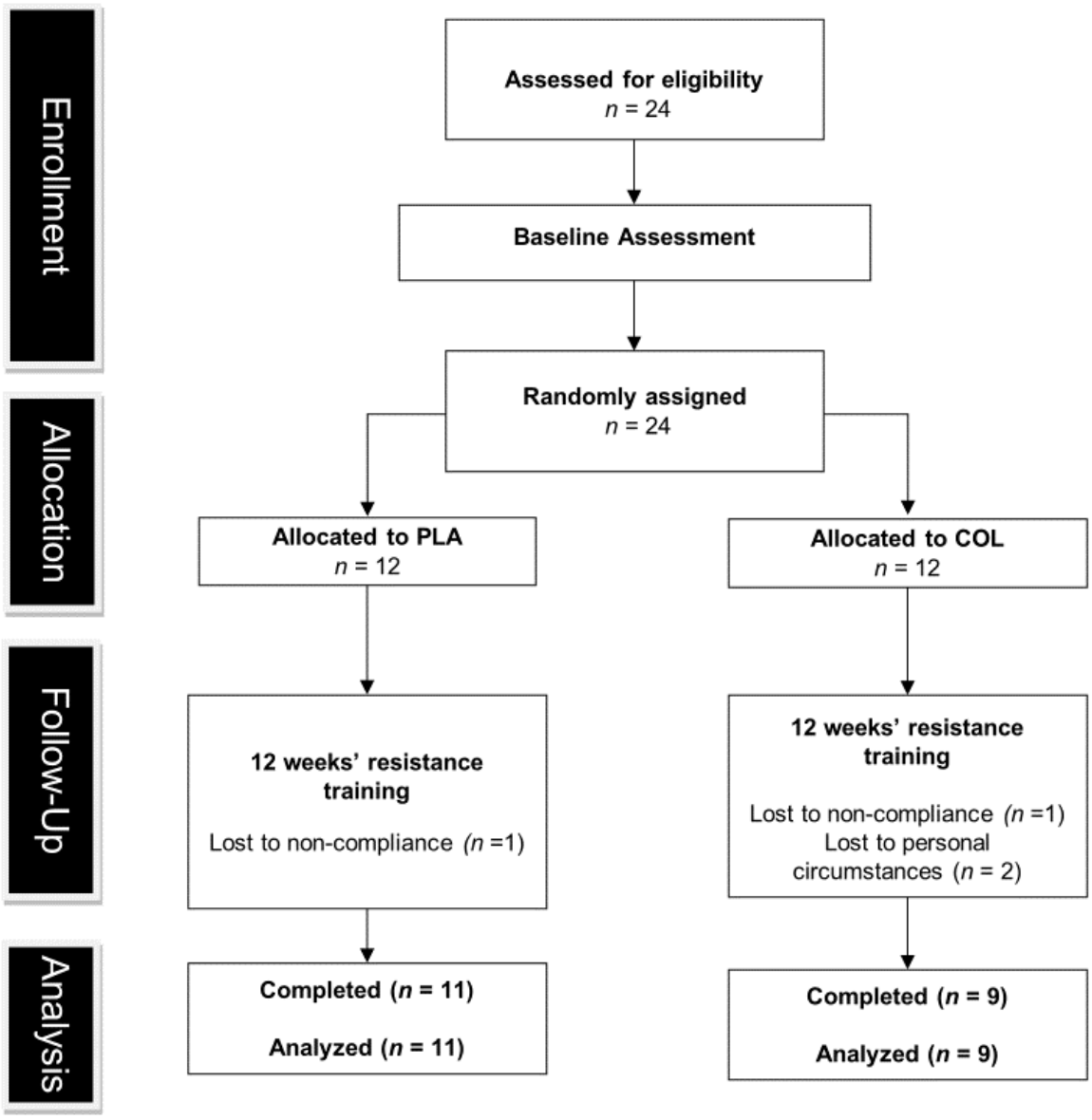


**Figure 1.** CONSORT flow diagram showing participant recruitment and intervention timeline. *PLA*, placebo group; *COL*, collagen group.

### *Habitual dietary behaviour and anthropometry*

Height was measured using a wall mounted stadiometer (Model 264, Seca Gmbh&Co. Hamburg, Germany) and body mass was measured using calibrated weighing scales (Model 704 W Seca Gmbh & Co., Hamburg, Germany), with participants shoeless and

wearing standard exercise clothing. During the familiarization and baseline testing period, participants were asked to record their habitual dietary behaviour using a food and drink diary across 3 days (Thursday to Saturday). Food diaries were analysed for total energy, macro- and micronutrient composition using professional dietary analysis software (Nutritics Research Edition, version 5.09, Dublin, Ireland). Total daily intakes were averaged across the three days and normalized to body mass. Absolute and relative (to body mass) daily nutritional compositions for COL and PLA are presented in Table 1.

***Muscle architecture***

B-Mode ultrasonography (Clear Vue 550, Koninklijke Philips, Eindhoven, The Netherlands) was used to obtain sagittal images of the *m. vastus lateralis* (VL) at 50% muscle length (Fig 2.). This muscle was chosen due to it being representative of the *m. quadriceps femoris* (25). Participants lay in a supine position on an elevated plinth, while all images were collected from the right leg with the knee and hip at full extension. The 38 mm wide 4–12 MHz linear transducer was placed on four sites of the skin of the right thigh to identify the origin and the distal myotendinous junction of the VL (to determine VL length); and the lateral and medial borders of the VL at 50% muscle length. Resting muscle architecture measurements (muscle thickness and fascicle pennation angle ($\theta_p$,), i.e. the angle of the fascicles as they insert into the lower aponeurosis) were then obtained by taking a single image of the centre of the VL muscle belly To prevent compression of the underlying muscle structures during ultrasound-based muscle architecture measurements, the transducer was positioned without contacting the skin, using a generous layer of ultrasound gel as the sole interface. Each image was manually analysed using image analysis software by a single operator with 10 years' ultrasound/ImageJ experience (Image J v1.8.0, National

Institute of Health, MD, USA). Muscle thickness (MT) was computed as the mean distance between the upper and lower aponeuroses, based on three perpendicular lines drawn at equal intervals along the width of the image. Fascicle pennation angle was computed from the mean angle of at least three fascicle insertions into the lower aponeurosis.

***Patellar tendon morphology***

Participants were firmly and securely strapped to an isokinetic dynamometer (Biodex System 3, IPRS, Suffolk, UK) in a seated position with their knee joint set at 90° knee flexion and their hip joint set at 85°. Patellar tendon (PT) morphology was assessed via B-mode ultrasound (Clear Vue 550, Koninklijke Philips, Eindhoven, The Netherlands) with the participant at rest. PT length was defined as the distance between the patella apex and insertion point of the PT into the tibial tuberosity, while the 38 mm wide linear transducer was placed over the knee in the sagittal plane. PT cross-sectional area was assessed at 3 points, 25%, 50%, and 75% tendon length, after these locations were identified with ultrasound and marked on the skin with an indelible pen. An axial scan to produce a transverse image was taken at each point ensuring all borders of the PT were clearly visible, which typically took 1 - 3 efforts per image. Each image was manually analysed by tracing the outline of PT using image analysis software (Image J v1.8.0, National Institute of Health, MD, USA).

***Patellar tendon mechanical properties***

*Maximal isometric strength*

Maximal isometric knee extension (KE) and knee flexion (KF) torque were assessed in the seated position, as described above. Participants were familiarized by performing 3 submaximal isometric KE and KF contractions. Participants were then

asked to perform between 2 and 4 KE and KF maximum voluntary contractions (MVCs) and asked to 'push' or 'pull' as hard as possible while receiving constant verbal encouragement. The number of attempts was increased if the highest attempt was ≥ 10% higher than the second highest attempt. Each MVC lasted ~2-3 s with 60 s rest between contractions. The highest KE MVC and KF MVC were used for analysis. The analogue torque signal was sampled at 2000 Hz and recorded using commercially available data acquisition software (EMGworks Acquisition v. 4.8.0, Delsys Inc., Manchester, UK). During offline analysis, the torque was low pass-filtered at 10 Hz, using a second order Butterworth filter, and corrected for gravity by subtracting the baseline torque. The torque was divided by the PT moment arm of healthy men at 90° knee flexion (Erskine *et al.*, 2009) in order to calculate force (N).

KE rate of torque development (RTD) was also calculated from the torque-time curve produced during MVC (where no countermovement could be detected). The time of torque onset was identified manually as the last trough in the baseline torque before an exponential increase. Torque at 50 ms, 100 ms, and 150 ms after torque onset was measured, and the RTD was calculated during 0-50, 50-100, and 100-150 ms time windows by dividing the difference in torque by the difference in time. Peak RTD (pRTD) was also calculated as the steepest slope in the torque-time curve occurring in the first 150 ms after torque onset. All RTD measures were then normalized by expressing them as a percentage of KE maximal isometric voluntary torque.

*Antagonist co-activation*

Hamstring activation was assessed via surface electromyography (sEMG) of the biceps femoris long head (BF) and was recorded during all contractions using a wireless EMG system (Trigno Wireless, Delsys Inc., Manchester, UK). The BF was

identified by palpation during submaximal knee flexion. The skin was prepared by shaving, abrading and cleaning with alcohol. A single wireless sensor (Trigno Avanti, Delsys Inc., Manchester, UK) was attached at 2/3 muscle length from the proximal end (Freriks *et al.*, 1999). To ensure synchronization between measures, EMG signals were sampled using the same analogue to digital converter and the same frequency as the torque signals. During analysis, EMG signals were band-pass filtered, using a second-order Butterworth filter at 10 – 500 Hz. The root mean square (RMS) of the filtered EMG signal was measured over a 500 ms epoch around maximal torque production during KF MVC. Additionally, the torque and EMG amplitude were resampled to match the ultrasound video frequency (28 – 38 Hz). During the ramped contraction, antagonist torque was estimated by expressing the KF BF amplitude relative to the BF amplitude during KF MVC and multiplying by the maximal KF torque.

*Force-elongation relationship and patellar tendon stiffness*

In order to precondition the PT, 10 repetitions of isokinetic knee extension and flexion through the participants full range of motion were performed at $60°\cdot s^{-1}$. The participant was instructed to perform the first repetition at approximately 10% of their perceived maximum effort and increase effort by 10% on every repetition before a final $10^{th}$ repetition performed at maximal (100%) effort. Ramped isometric KE MVCs (RMVCs) were used to measure the PT force-elongation relationship and calculate its mechanical properties during KE. The 38 mm wide linear array transducer (Model L12-4 [4 -12 MHz] Phillips ClearVue 550, Amsterdam, NL) was not wide enough to capture the entire length of the PT in one image. Therefore, a 2 mm wide strip of surgical tape (3M™ Transpore™ White Medical Tape, 3M, Dublin, Ireland) was placed transversely over the skin to act as an echo-absorbent reference point at 50%

tendon length. The probe was placed in the sagittal plane over the PT and held firmly in place but with minimal pressure (to avoid compressing the tendon) during each contraction. Participants performed between 2 and 5 submaximal ramped contractions until the investigator was satisfied that they could perform a smooth contraction with a constant loading rate. Participants then performed 2-3 RMVCs lasting 6 s. This process was repeated with the probe placed on the distal aspect of the PT, with both the tape and tibial tuberosity clearly visible in the video image. PT elongation was measured offline, using open-source semi-automatic tracking software (Tracker for Windows v.6.0.8) to track displacement of both the PT apex, and the PT insertion on the tibial tuberosity. In cases where lower-quality frames caused the semi-automatic tracker to lose the pixel, these frames (<10% of ~150 per video) were manually excluded following qualitative inspection. Concurrently, KE torque, antagonist sEMG were recorded as stated above, with an additional EMG sensor attached to the ultrasound probe to provide a simultaneous signal interruption on both the ultrasound video and torque trace, allowing time synchronization between measurements during offline analysis.

To synchronize the force and elongation measurements during the proximal and distal ultrasound videos and contractions, force-time curves were resampled in the first instance to match the sampling frequency of each ultrasound video. Separate force-elongation curves were then constructed for the proximal and distal PT. The force readings from the weakest of the two RMVC measurements were used for analysis. Total PT elongation was calculated by summing the elongation at one end during the weakest RMVC and the interpolated elongation from the opposing end at matching force levels. For example, if proximal elongation at 1600 N was 1.5 mm, the corresponding distal elongation at 1600 N (e.g., 0.5 mm) was interpolated from the

distal force-elongation curve and summed to calculate the total elongation (2.0 mm). Force was calculated from torque, as described during the maximal isometric contraction section above, and each force-elongation relationship was fitted with a second or third order polynomial (Figure 2). For each participant, patellar tendon stiffness ($K$) was calculated from the force-elongation slope, using the highest 20% absolute force values of the weakest RMVC (typically obtained pre-training). Young's modulus ($\mathcal{E}$) was calculated by obtaining the ratio of PT length to mean PT CSA and multiplying by the PT stiffness. PT stress was calculated by dividing maximal PT force by mean PT CSA. PT strain was expressed as a percentage of maximal PT elongation relative to PT resting length.

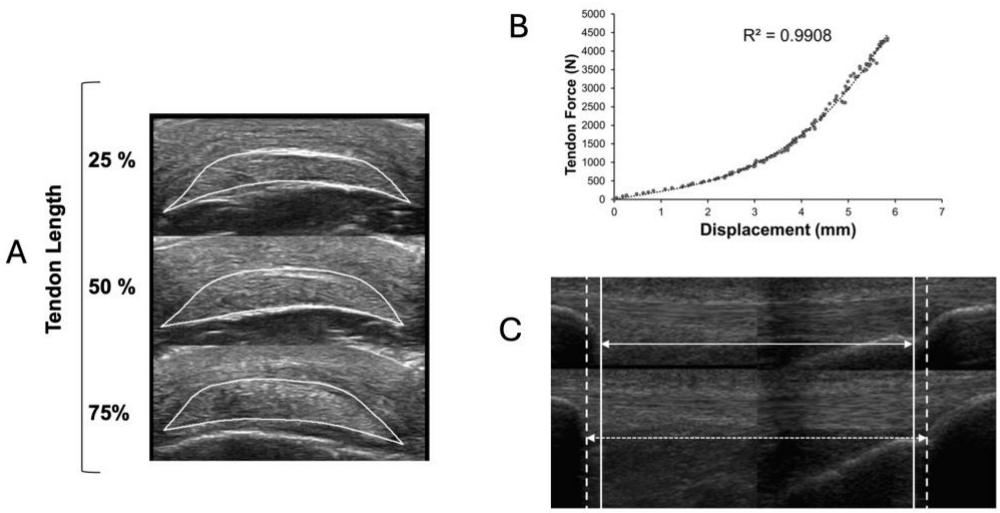


**Figure 4. A.** Representative ultrasound image of patellar tendon (PT) cross sectional area at 25, 50, and 75 % tendon length. **B.** Example of the force-elongation curve during the 6-second ramped isometric maximum voluntary contraction (MVC) and **C.** Representative ultrasound image depicting changes in PT length from rest to MVC (solid arrow represents resting tendon length; dashed arrow represents tendon length at MVC).

***Bilateral vertical countermovement jump (CMJ) and broad jump (BJ)***

Participants performed an additional dynamic warm-up consisting of 5 min submaximal jogging activity, 5 min dynamic stretching, and 3 submaximal CMJs and BJs. For CMJ, participants were required to remove shoes and stand upright with hands remaining on hips for the entirety of the jump. They were then instructed to quickly squat to a self-selected depth before immediately and rapidly reversing the movement into a maximal effort jump with knees fully extended. To ensure consistent jump height measurements using photoelectric cells (Optojump, Mircrogate, Bolanzo, Italy) and that participants jumped and landed in the same spot, tape marked with an 'X' was placed on the floor as a visual reference. During each visit, participants performed 3–5 practice submaximal jumps, receiving live feedback to reinforce proper technique. Instructions emphasised landing with knees extended and minimizing excessive flexion upon landing. Valid and invalid trials were demonstrated during the familiarisation session and prior to each assessment. Three maximal CMJs were performed, separated by 1 min rest, with jump height assessed. BJs required the same start position as CMJ, and participants were asked to jump forward as far as possible, keeping the hands on the hips and land with both feet. BJ distance was measured by the placing a wooden dowel against the heels to mark the landing spot and aligned with a measuring tape on the floor. Three maximum BJ efforts were performed and separated by 1 min rest. The highest CMJ and longest BJ were used for analysis.

***Barbell back squat 10-Repetition Maximum (10-RM)***

Participants rested for 2 min after completing all jump assessments. In order to standardize depth during back squats, participants were asked to place the 20 kg Olympic barbell on their shoulders and squat down until they reached a depth

equivalent to 90° knee flexion, which was determined by the researcher using a goniometer. A box was placed at this height to allow participants to touch briefly on each repetition. One set of the barbell back squats with no additional load was performed initially, followed by 1 set of 10 repetitions at 50% of their estimate 10 RM. The load was then increased by 5 – 20 kg on each subsequent set for a total of 4 – 6 attempts, separated by 3 min rest until the participant could only perform a maximum of 10 repetitions. 10-RM was deemed to be the load used during the final set where the participant successfully completed 10 repetitions.

***Resistance training***

All participants completed two resistance training sessions per week (separated by 48 – 72 h) for 12 weeks, resulting in a total of 24 sessions (100% participant compliance), with the aim of overloading the lower limbs, particularly the quadriceps MTU. Participants began the first training session working at a load of 90% 10-RM. The objective during each session was to complete at least 8, but no more than 10 repetitions for all 4 sets, each set separated by 3 min rest, with participants instructed to terminate sets at 10 repetitions or concentric failure depending on which came first. Participants were instructed to perform the eccentric portion under control over ~ 2 s duration, and the range of motion was standardised for each exercise, with participants instructed to lightly touch a tactile indicator (e.g., weight bench and plates for squat depth). Each exercise was progressed independently, and load was determined by performance in the same session the previous week, i.e. external load was increased by 2.5 – 5 kg when the participant could successfully complete 4 sets of 10 repetitions. This procedure applied to all exercises for the first 6 weeks' training. For the second 6 weeks, all exercises and rest periods remained the same, however, the objective for each session was to complete at least 6 repetitions but no more than 8 repetitions

during all 4 sets. This was done to ensure continuous overload and limit the chance of plateauing in volume and intensity. Participants then progressed each exercise by adding 2.5 – 5 kg each week after successful completion of 4 sets of 8 repetitions during the corresponding session during the previous week.

***Nutritional supplementation***

The dry ingredients for both the hydrolysed collagen supplement (COL) and maltodextrin placebo (PLA) were prepared into sachets by a laboratory technician independent of the study. The sachets were stored in containers identifiable by red and green coloured labels, and matched pairs of participants were placed into "Red" and "Green" groups, to ensure double blinding of the participants and investigators. The composition of each supplement is included in supplementary figure 1 (10.6084/m9.figshare.27094399). The COL contained 30 g unflavoured hydrolysed collagen (Collagen Protein, MyProtein, Manchester, UK), 50 mg vitamin C powder (Holland & Barrett, Dublin, Ireland) and 3 g non-caloric sweetener (Pure Via 100% Xylitol, Mersiant UK LTD., Buckinghamshire, UK). The PLA was matched for caloric value, vitamin C content, taste, and colour and contained 30.5 g of maltodextrin (MyProtein, Manchester, UK), 50 mg vitamin C powder, and 4 g of non-caloric sweetener. As soon as each training session ended, participants were instructed to collect the coloured sachet of dry supplement ingredients from the sample box corresponding to their colour group allocation. Each participant was provided with their own opaque bottle and mixed the dry ingredients with 400 mL water, which they consumed under supervision within 5 min of completing their training session. Supplement intake only took place on training days and was supervised to ensure compliance. Outside of the supervised training sessions, participants were instructed to maintain their habitual dietary habits for the duration of the study.

***Statistical Analysis***

Data are reported as mean ± standard deviation unless stated otherwise. All data were analysed using the statistical software package for social sciences (SPSS v. 28, IBM, Armonk NY, USA), with significance accepted at $P<0.05$. Independent t-tests were performed on all variables between COL and PLA groups to assess whether there were any differences at baseline. Adaptations to the training and nutrition intervention were assessed for main effects and training × group interaction effects by two-way analysis of variance (ANOVA). When significant main effects or interaction effects were observed, paired t-tests were used for post hoc pairwise comparisons. Independent t-tests were used to compare the absolute pre-to-post differences between groups. Changes in PT CSA were assessed for main effects of training and location (i.e. the location along its length relative to tendon origin), as well as any interactions between training, group, and location by three-way ANOVA, with paired t-tests used for post hoc pairwise comparisons. Partial eta squared ($\eta_p^2$) for ANOVA effects and Cohen's d for t-tests were reported as effect size estimates for each corresponding statistical model. The thresholds for $\eta p^2$ and Cohen's d are categorized as small ($\eta_p^2 = 0.01$, d = 0.20), medium ($\eta_p^2 = 0.06$, d = 0.50), and large ($\eta_p^2 = 0.14$, d = 0.80) (Cohen, 2013).

# 7.3 Results

***Dietary intake and body mass***

There were no differences in absolute intake nor intake relative to body mass of macronutrients or vitamin C between COL and PLA during the pre-intervention period (Table 1, $P > 0.05$). There were no differences in body mass between groups pre-

intervention ($t_{18}$ = 1.16, P = 0.261) and there were no changes following the intervention ($F_{1,18}$ = 2.87, P = 0.109).

**Table 1.** Habitual energy, macronutrient, and micronutrient intake assessed during the pre-training period.

| Nutritional Composition | COL (n = 9) | PLA (n = 11) | t-test, P |
|---|---|---|---|
| *Energy intake* | | | |
| Habitual intake (kcal·d$^{-1}$) | 1934 ± 648 | 2238 ± 494 | 0.303 |
| *Carbohydrate Intake* | | | |
| Habitual intake (g·d$^{-1}$) | 218.9 ± 112.3 | 238.7 ± 49.3 | 0.641 |
| Habitual intake (g·kg·d$^{-1}$) | 2.8 ± 10.9 | 2.8 ± 4.8 | 0.675 |
| *Protein Intake* | | | |
| Habitual intake (g·d$^{-1}$) | 81.6 ± 22.7 | 92.6 ± 19.1 | 0.314 |
| Habitual intake (g·kg·d$^{-1}$) | 1.1 ± 2.2 | 1.1 ± 1.9 | 0.328 |
| *Fat intake* | | | |
| Habitual intake (g·d$^{-1}$) | 70.9 ± 19.4 | 84.3 ± 49.3 | 0.357 |
| Habitual intake (g·kg·d$^{-1}$) | 0.9 ± 1.9 | 1.0 ± 3.2 | 0.606 |
| *Vitamin C intake* | | | |
| Habitual intake (mg·d$^{-1}$) | 114.6 ± 36.5 | 108.6 ± 20.4 | 0.901 |
| Habitual intake (mg·kg·d$^{-1}$) | 1.5 ± 3.6 | 1.3 ± 11.8 | 0.618 |

***Muscle strength, power and architecture***

At baseline, there were no differences in muscle KE MVT ($t_{18}$ = 0.323, P = 0.751) or pRTD ($t_{18}$ = -.197, P = 0.846) between groups. Changes in isometric strength, VL muscle architecture, and jump performance following 12 weeks' RT are presented in Table 2. All variables except for VL fascicle pennation angle improved following the intervention (P < 0.05) but there were no group × time interactions for any of the measures (P > 0.05).

**Table 2.** Muscle function and architecture adaptations to 12 weeks' resistance training. Data are mean ± SD.

| | **PLA** | | **COL** | | |
|---|---|---|---|---|---|
| **Variable** | **Pre** | **Post** | **Pre** | **Post** | **G × T, *P*** |
| KE MVT (N·m) | 232 ± 56 | 247 ± 46 * | 213 ± 62 | 233 ± 44 * | 0.754 |
| 10-RM (kg) | 67 ± 16 | 97 ± 18* | 72 ± 14 | 109 ± 19* | 0.116 |
| KE pRTD ($N \cdot m.s^{-1}$) | 990 ± 479 | 1215 ± 404* | 1038 ± 582 | 1211.7 ± 577* | 0.637 |
| KE pRTD (% MVT) | 411 ± 160 | 500 ± 155* | 468 ± 238 | 532 ± 240* | 0.581 |
| BJ distance (cm) | 140 ± 30 | 143 ± 32* | 149 ± 29 | 156 ± 26* | 0.552 |
| CMJ height (cm) | 25.1 ± 5.2 | 26.9 ± 6.4* | 26.3 ± 5.6 | 28.7 ± 6.8* | 0.589 |
| CMJ Power (W) | 3272 ± 626 | 3382 ± 689* | 3065 ± 499 | 3221 ± 563* | 0.422 |
| VL MT (mm) | 24.8 ± 3.2 | 25.1 ± 2.4* | 23.4 ± 3.2 | 24.5 ± 2.9* | 0.714 |
| VL $\theta$p (°) | 16.2 ± 3.1 | 16.8 ± 2.9 | 16.6 ± 2.8 | 17.2 ± 1.5 | 0.934 |

*KE,* knee extension; *MVT,* maximum voluntary torque; *10-RM,* leg press 10 repetition maximum; *pRTD,* peak rate of torque development; *BJ,* broad jump; *CMJ,* countermovement jump; *VL,* vastus lateralis; *MT,* muscle thickness; $\theta_p$, fascicle pennation angle; *Greater than pre-training *(*$P < 0.05$).

***Explosive torque production and rate of torque development (RTD)***

Absolute and normalized explosive torque production at 50, 100, and 150 ms after the onset of torque were not different between COL and PLA at baseline ($P > 0.05$). Similarly, there were no between group differences in absolute or normalized pRTD at baseline ($t_{17} = -.197$, P =0 .846; $t_{17} = -.197$, $P = 0.846$), which occurred at 78 ± 20 ms after torque onset. Increases in pRTD post-intervention are shown in Table 2 but there was no group × time interaction ($F_{1,17} = 0.231$, $P = 0.637$). Both absolute (time, $F_{1,17} = 6.366$, $P = 0.022$, $\eta_p^2 = 0.272$) and relative (time, $F_{1,17} = 6.6719$, $P = 0.020$, $\eta_p^2 = 0.294$) RTD increased following the intervention but there was no group × time interaction ($F_{1,17} = 0.141$, $P = 0.712$). There was also an effect of time window, where RTD was highest 50 – 100 ms after torque onset ($F_{1,17} = 34.077$, $P < 0.001$, $\eta_p^2 = 0.667$).

Absolute explosive torque increased at 100 ms ($F_{1,17} = 15.022$, $P = 0.001$, $\eta_p^2 = 0.484$) and 150 ms ($F_{1,17} = 8.543$, $P = 0.010$, $\eta_p^2 = 0.348$) after the onset of torque following the 12-weeks' RT, respectively but not at 50 ms after torque onset ($F_{1,17} = 4.259$, $P = 0.056$). Normalized explosive torque increased post training at 100 ms ($F_{1,17} = 5.672$, $P = 0.030$, $\eta_p^2 = 0.262$) but not at 50 ($F_{1,17} = 1.226$, $P = 0.285$) nor 150 ms ($F_{1,17} = 2.894$, $P = 0.108$) after torque onset (Figure 3).

However, for absolute and normalized RTD, there were no interactions between time × time window, or time × time window × group ($P > 0.05$), and for absolute and

normalized explosive torque and RTD there were no interactions between time × group, and ($P > 0.05$). Pre- to post- training changes in explosive torque and RTD are displayed in Figure 4.

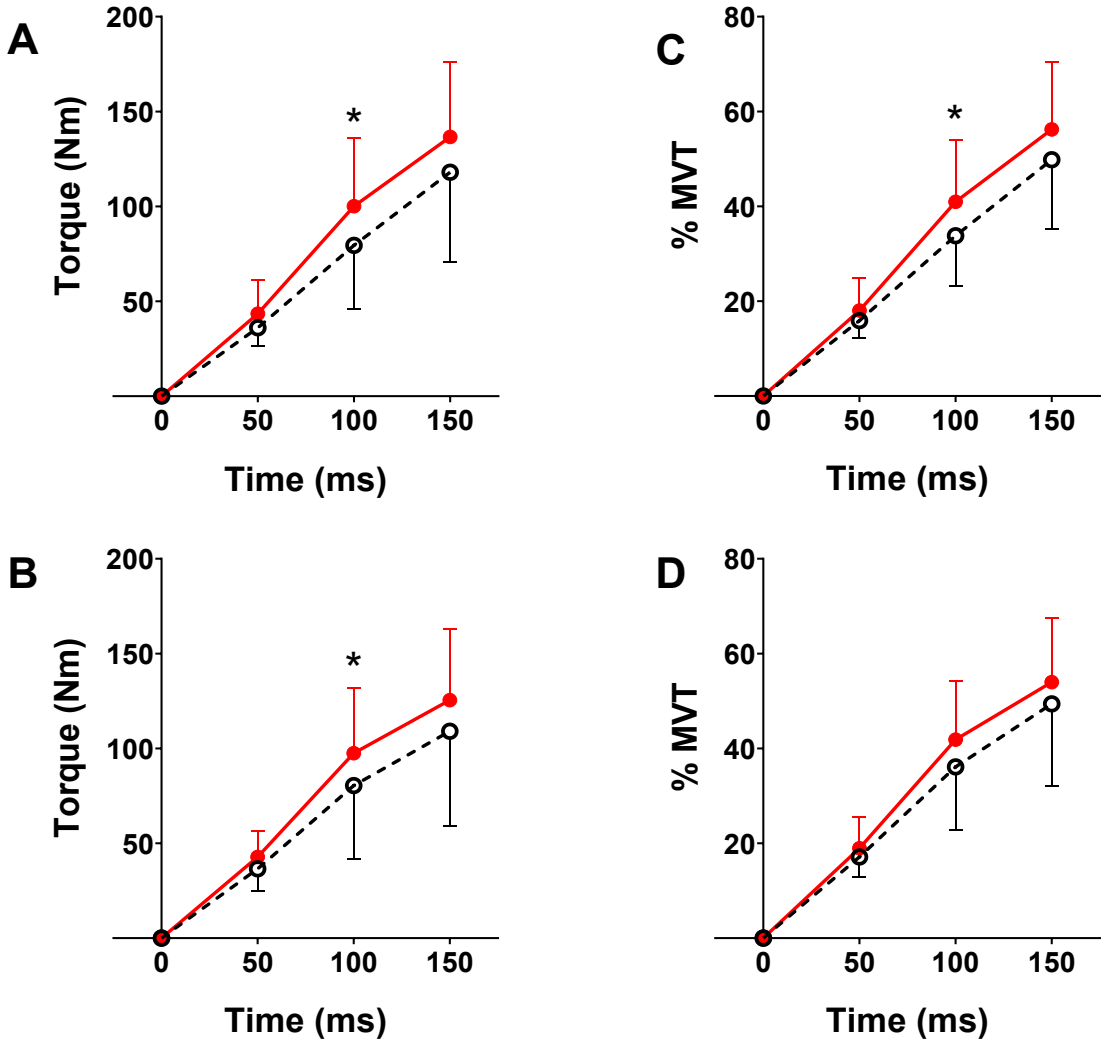


**Figure 3.** Absolute explosive torque before (dashed line) and after (red line) 12 weeks' resistance training without (**A**, PLA, n = 10) and with 30 g hydrolysed collagen supplementation (**B**, COL, n = 9); and explosive torque normalized to maximum voluntary torque (MVT) before (dashed line) and after (red line) 12 weeks' resistance training without (**C**, PLA, n = 10) and with 30 g hydrolysed collagen supplementation (**D**, COL, n = 9).

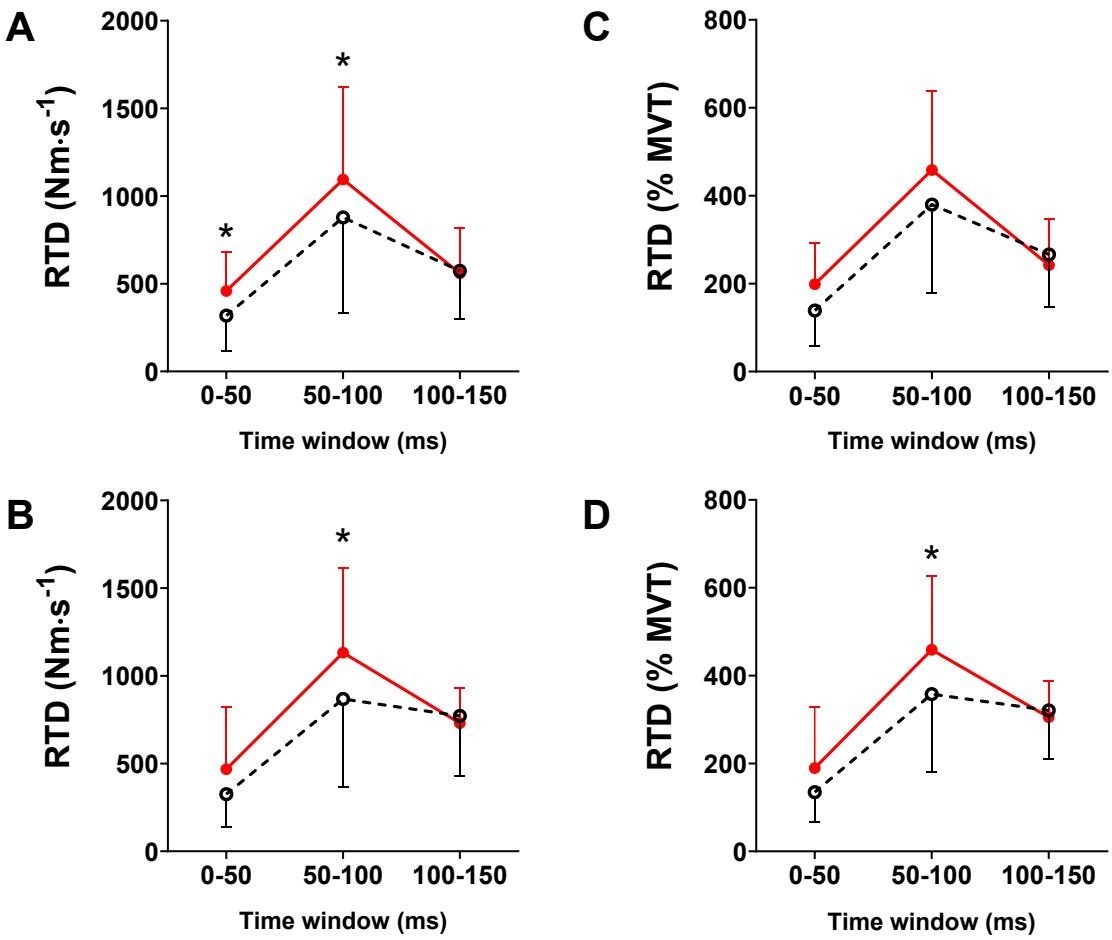


**Figure 4.** Absolute rate of torque development (RTD) before (dashed line) and after (red line) 12 weeks' resistance training with (**A**, COL = 9) without 30 g hydrolysed collagen supplementation (**B**, PLA, n = 10); normalized RTD before (dashed line) and after (red line) 12 weeks' resistance training with (**C**, COL, n = 9) and without 30 g hydrolysed collagen supplementation (**D**, PLA, n = 10).

***Patellar tendon cross sectional area***

At baseline, there were no between group differences in mean PT CSA (P = 0.064). However, CSA was not the same at each location along tendon length [main location effect ($F_{2,38} = 40.956$, $P < 0.001$, $\eta_p^2 = 0.789$)], and post-hoc analysis revealed that CSA was larger at 25% ($CSA_{25\%}$) and 75% ($CSA_{75\%}$) of its length compared with 50%

($CSA_{50\%}$) ($P < 0.001$), and larger at $CSA_{25\%}$ compared to $CSA_{75\%}$ ($P = 0.035$). Training increased mean PT CSA ($F_{1,18} = 9.948$, $P = 0.001$, $\eta_p^2 = 0.443$) and there was a time × group interaction ($F_{1,18} = 6.037$, $P = 0.024$, $\eta_p^2 = 0.244$, Figure 4). In COL, PT $CSA_{25\%}$ and PT $CSA_{75\%}$ increased by 6.6 ± 7.3 $mm^2$ ($P = 0.03$, $d = 0.901$, $d = 1.1$) and 6.0 ± 5.4 $mm^2$ ($P = 0.01$), respectively, with no change at PT $CSA_{50\%}$ ($P = 0.15$) after 12 weeks' RT. There were no changes from pre- to post- training at any location along the PT in PLA ($F_{1,20} = 0.404$, $P = 0.539$, Figure 5).

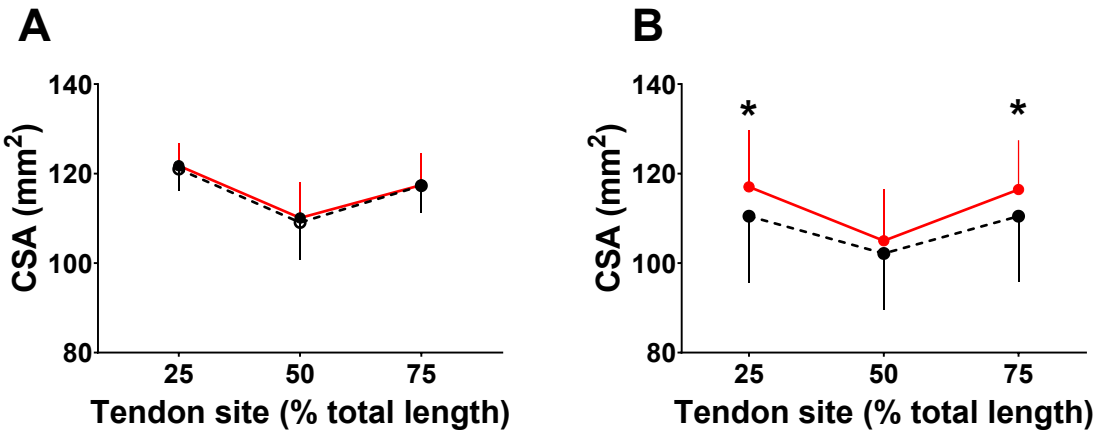


**Figure 5.** Patellar tendon cross sectional area ($mm^2$) before (black circles, dashed line) and after (red circles, red line) 12 weeks' resistance training in **A**. PLA (n = 11) and **B**. COL (n = 9). * Greater than pre ($P < 0.05$).

***Patellar tendon mechanical properties***

PT stress was higher in COL compared to PLA at baseline ($t_{18} = 2.13$, $P = 0.047$). There was a main effect of time ($F_{1,18} = 35.04$; $P < 0.001$) where PT stress increased post-training, but no effect of group ($F_{1,18} = 2.16$; $P = 0.159$), and no group × time interaction ($F_{1,18} = 2.97$, $P = 0.102$). There was a main effect of time on PT strain, which decreased post-training ($F_{1,18} = 4.82$, $P = 0.041$, $\eta_p^2 = 0.443$), however, there

was no interaction ($F_{1,18}$ = 3.38, P = 0.082). The mechanical properties of the patellar tendon are available in Table 3.

**Table 3.** Patellar tendon mechanical properties before (Pre) and after (Post) 12-weeks' RT with (COL) and without (PLA) hydrolysed collagen supplementation.

| | **PLA** | | **COL** | | |
|---|---|---|---|---|---|
| **Variable** | **Pre** | **Post** | **Pre** | **Post** | **G × T, *P*** |
| **Stiffness (N/mm)** | 1304 ± 622 | 1551 ± 612 | 1174 ± 294 | 1836 ± 518 | 0.009 |
| **Young's Modulus (GPa)** | 0.53 ± 0.29 | 0.62 ± 0.29 | 0.52 ± 0.14 | 0.76 ± 0.17 | 0.018 |
| **Stress (MPa)** | 25.5 ± 8.5 | 42.1 ± 9.6* | 34.2 ± 9.6 | 43.3 ± 7.3* | 0.103 |
| **Elongation (mm)** | 4.8 ± 1.7 | 4.6 ± 1.2 | 5.7 ± 1.8 | 4.6 ± 1.1* | 0.053 |
| **Strain (%)** | 10.7 ± 4.0 | 9.9 ± 2.6 | 11.3 ± 3.4 | 9.1 ± 2.3* | 0.082 |
| **Mean tendon CSA (mm²)** | 117 ± 6 | 117 ± 6 | 108 ± 13 | 115 ± 10* | 0.027 |
| **RMVC peak tendon force (N)** | 2956 ± 990 | 3658 ± 1041* | 3408 ± 1026 | 3963 ± 1157* | 0.288 |

Data are mean ± SD. *PLA:* placebo group; *COL:* hydrolysed collagen group. *Different to pre-training ($P < 0.05$).

Regarding patellar tendon stiffness ($k$), there were no differences between groups at baseline (P = 0.78). Following the intervention, however, there was a main effect of time ($F_{1,18} = 40.71$; P < 0.001, $\eta_p^2 = 0.693$), no main effect of group ($F_{1,18} = 0.113$, P = 0.741), but there was a group × time interaction ($F_{1,18} = 8.46$, P = 0.009, $\eta_p^2 = 0.320$). Post-hoc paired t-tests revealed that $k$ increased in both COL (P < 0.001, $d = 2.0$) and PLA (P = 0.019, $d = 0.811$), while an independent t-test on the change from pre- to post training revealed that the increase in $k$ was greater in COL ($k$, + 661 ± 331 N/mm) compared with PLA (247 ± 305 N/mm) ($t_{18} = 2.91$, P = 0.009, , $d = 1.31$, Figure 6).

Similarly, there was a main effect of time on Young's modulus ($\mathcal{E}$) ($F_{1,1}8 = 30.73$, P < 0.001, $\eta_p^2 = 0.631$), no main effect of group ($F_{1,18} = 0.398$, P = 0.536), but there was a group × time interaction ($F_{1,18} = 6.71$, P = 0.018, $\eta_p^2 = 0.271$, Figure 6), where $\mathcal{E}$ increased by 0.21 ± 0.13 GPa in COL (P < 0.001, $d = 1.83$), and by 0.09 ± 0.13 GPa in PLA (P = 0.041, $d = 0.662$). The pre- to post-training changes were greater in COL compared with PLA ($t_{18} = 2.91$, P = 0.048, $d = 1.16$).

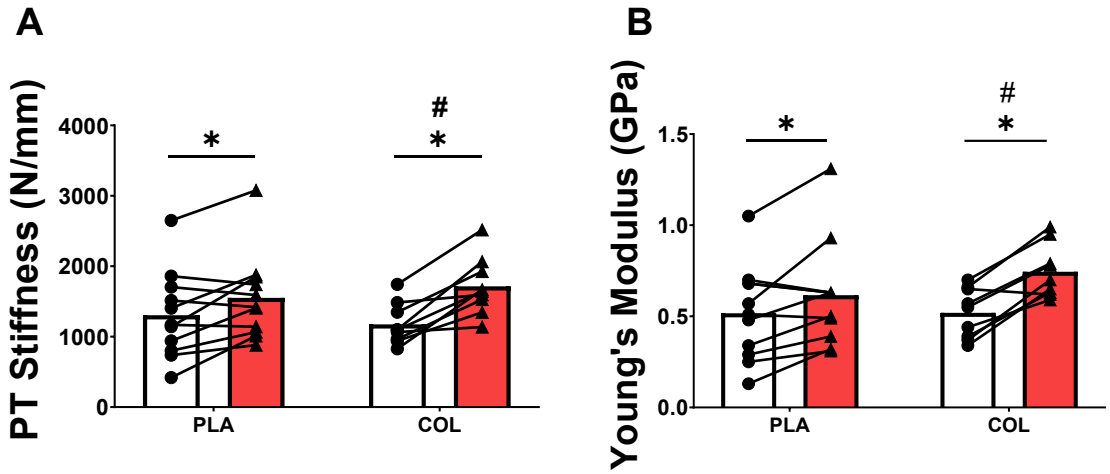


**Figure 6. A.** Patellar tendon stiffness (N/mm), and **B.** Young's modulus (GPa) before (white bar, circles) and after (red bar, triangles) 12 weeks' resistance training in COL (n = 9) and PLA (n = 11). * Greater than pre, # group × time interaction ($P < 0.05$).

# 7.4 Discussion

The objective of this study was to determine if supplementation with vitamin C-enriched hydrolysed collagen augmented changes in PT morphological and mechanical properties in healthy, recreationally active, middle-aged men. Consuming 30 g hydrolysed collagen enriched with 50 mg vitamin C twice per week after RT led to greater increases in PT CSA, stiffness and Young's modulus compared to RT alone. This study is the first to demonstrate the effects of high intensity RT on tendon adaptations in middle-aged men, and that these effects are enhanced by collagen supplementation.

The effects of RT on tendon stiffness are well established in young and old populations (Bohm *et al.*, 2015; Lazarczuk *et al.*, 2022). However, there is a gap in research concerning the effects of RT on tendon properties in *middle-aged* individuals. To our knowledge, only one study has examined these effects in middle-aged women,

demonstrating no change in tendon stiffness with low-load RT, specifically body weight squats (Kubo *et al.*, 2003). Furthermore, the combined influence of nutrition and high-intensity RT on tendon mechanical properties in aging populations remains unexplored. Therefore, our study represents the first investigation into these effects in *middle-aged* men.

In our study, participants in the placebo group (PLA) exhibited an increase in tendon stiffness without a corresponding increase in tendon size. This finding diverges from previous research in young populations, where RT-induced tendon hypertrophy has consistently been observed (Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009; Massey *et al.*, 2017; Quinlan *et al.*, 2021). Notably, Quinlan et al. (Quinlan *et al.*, 2021), reported similar findings in older males, wherein patellar tendon mechanical properties improved without an increase in cross-sectional area (CSA) following eight weeks' RT, mirroring the outcomes in our middle-aged PLA group, who underwent RT without COL. Conversely, younger males in the same study experienced both tendon hypertrophy and increased stiffness (Quinlan *et al.*, 2021). The increase in tendon stiffness observed in aging individuals may be attributed to alterations in material properties, such as increased crosslinking and/or collagen fibril density (Heinemeier & Kjaer, 2011) rather than tendon hypertrophy.

A meta-regression analysis of studies that concurrently assessed tendon material properties and CSA suggested that an increase in modulus was the main contributor to short term changes in stiffness (Lazarczuk *et al.*, 2022). However, the authors included various modes of chronic exercise with different contraction types and intensities, whereas *high intensity* RT (like that employed in our study design) is known to be the key driver of tendon hypertrophy (Bohm *et al.*, 2015). Conversely, we observed augmented tendon hypertrophy alongside enhanced stiffness in COL and, as reported

previously, the hypertrophy occurred at the proximal and distal locations, where the tendon is closest to the musculotendinous- and osteotendinous- junctions, respectively (Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009). This suggests that the tendon hypertrophy in COL was the primary factor contributing to the greater increase in stiffness.

RE stimulates collagen synthesis in tendon (Miller *et al.*, 2005a; Crossland *et al.*, 2023), while collagen supplementation increases blood concentration of collagen amino acids (Shaw *et al.*, 2017; Alcock *et al.*, 2019b; Skov *et al.*, 2019; Lee *et al.*, 2023c). Crucially, Lee at al. (Lee *et al.*, 2023c) and in Chapter Four of this thesis have shown that ingesting 30 g HC prior to RE increased whole body collagen synthesis in the hours following RE in both young and middle-aged men. The greater bioavailability of key collagen amino acids may have stimulated greater whole body collagen synthesis by either supplying the crucial amino acids following a RE-induced increase in collagen synthesis, or by stimulating collagen synthesis independently of RE via different signalling pathways (Mousavizadeh *et al.*, 2020), or both. The PT hypertrophy in the COL group, compared to none in the PLA group, can be attributed to the different collagen synthesis responses in middle-aged (Chapter Four) compared to young, resistance-trained men (Lee *et al.*, 2023c). Firstly, middle-aged men show no whole-body collagen synthesis response to a single bout of RE (Chapter Four). However, 15 g HC ingestion seems to overcome this anabolic resistance in middle-aged men, with 30 g HC having an even greater effect. This contrasts with young men, who show a clear augmentation in collagen synthesis following RE even without prior ingestion of HC (Lee *et al.*, 2023c). However, 15 g HC ingestion seems to overcome this anabolic resistance in middle-aged men, with 30 g HC having an even greater effect. This lack of collagen synthesis increase in middle-aged men without HC

ingestion may explain the absence of PT hypertrophy in our PLA group. In contrast, the COL group showed a 5.2 ± 4.9 percentage increase in PT CSA, likely due to enhanced net collagen turnover from regular key amino acid ingestion in the COL group.

Two related studies suggest that collagen peptide supplementation combined with RT augments both patellar and Achilles tendon hypertrophy, with concomitant increases in patellar tendon stiffness (Jerger *et al.*, 2023) but not Achilles tendon stiffness (Jerger *et al.*, 2022). Our study's distal PT hypertrophy in the COL group (+6.0 ± 5.2%) is less than the 10.7% increase reported by Jerger and colleagues (Jerger *et al.*, 2023) in their collagen supplementation group but similar to the 6.5% increase in their RT-only group. Total weekly training volume for the knee extensors in the study by Jerger and colleagues (24), which involved younger participants, was similar to our study. Thus, age differences in study populations likely account for these discrepancies. Without collagen supplementation, older men may not experience the same hypertrophy from RT as younger men (Quinlan *et al.*, 2021). Our data support the notion that tendon hypertrophy is blunted with ageing, starting in middle-age, but 30 g hydrolysed collagen can restore the tendon's ability to hypertrophy in response to RT. Finally, Balshaw *et al.* (2022) reported increased PT stiffness in young untrained men after 14-weeks' RT, with no additional effects from 15g·d$^{-1}$ collagen ingestion, aligning with findings that 15 g collagen is insufficient to increase collagen synthesis after acute resistance exercise in young men, while 30 g does (Lee *et al.*, 2023c).

In young, female soccer players, both high-intensity resistance training bodyweight training and combined with 30 g collagen supplementation resulted in 15% and 18% in patellar tendon stiffness respectively in academy and professional athletes (Lee *et al.*, 2023a; Lee *et al.*, 2024a). By comparison, the observed 56 ± 29 % increase in

stiffness in our collagen group appears high. However, given that our participants were untrained prior to the study [unlike the athletes in both studies by Lee and colleagues (Lee *et al.*, 2023a; Lee *et al.*, 2024a)], and the lower tendon stiffness of the middle-aged men at baseline, it is likely they had greater capacity for adaptation compared to the younger athletes. Furthermore, our data align with the magnitude of change in tendon stiffness and Young's modulus observed in previously untrained older men (Reeves *et al.*, 2003a; Quinlan *et al.*, 2021). Additionally, it would be expected that *high intensity* RT would result in greater overload of the muscle-tendon unit, leading to greater strain on the tendon and thus a stronger stimulus for adaptation (Lavagnino *et al.*, 2003; Arampatzis *et al.*, 2007).

Our finding that RT alone increased muscle thickness is consistent with the body of research on adaptation to RT (Schoenfeld *et al.*, 2017), yet there was no additional benefit of HC supplementation. In contrast, three recent studies have suggested muscle hypertrophy is enhanced by collagen supplementation (albeit in a very small number of measures) (Balshaw *et al.*, 2022; Jerger *et al.*, 2022). Although one study found that 15 g collagen protein can upregulate cellular pathways associated with muscle protein synthesis (Centner *et al.*, 2022b), the notion that collagen protein may augment muscular adaptations to RT is highly speculative. The amino acid profile of collagen is different to that of high quality proteins such as whey, which has a relatively high composition of essential amino acids known to stimulate muscle protein synthesis (MPS) independently of RE (Tang *et al.*, 2009). It is this difference in amino acid composition that probably explains why whey protein is a superior stimulant of MPS than collagen (Oikawa *et al.*, 2020). This has also been demonstrated over time, whereby whey has produced superior outcomes to collagen after chronic RT (Jacinto *et al.*, 2022a). The habitual protein intake of participants in our study (1.1 $g \cdot kg^{-1}$

[before 30 g HC supplementation]) may have been insufficient to support optimal hypertrophy in this population compared with intakes ≥ 1.6 g·kg$^{-1}$ (Morton *et al.*, 2018; Nunes *et al.*, 2022). Even though our HC supplement increased daily protein intake by ~ 37 % on training days in COL, this did not augment muscle hypertrophy, which further supports the notion that collagen is inferior to other protein sources to promote *muscle* adaptation to RT.

The increases in strength and power (e.g. isometric strength and RTD, 10-RM back squat, CMJ and broad jump) observed in both groups can be explained by the effects of high intensity RT alone (Erskine *et al.*, 2010). Supplementation with 30 g hydrolysed collagen did not augment adaptations in any of these tasks, which is consistent with recent reports in young men (Balshaw *et al.*, 2022; Jerger *et al.*, 2022), yet conflicts with studies in older men where collagen supplementation outperformed placebo in certain measures of strength (Zdzieblik *et al.*, 2015; Zdzieblik *et al.*, 2021). Interestingly, while COL supplementation led to larger improvements in tendon stiffness and modulus compared to RT alone, these changes were not accompanied by additional gains in explosive strength, power, or RTD. This suggests that COL supplementation did not confer a further advantage over RT alone for improving RTD. A stiffer tendon should improve RTD and therefore translate to better dynamic exercise performance (Bojsen-Møller *et al.*, 2005; Maffiuletti *et al.*, 2016). Although the intrinsic properties of tendon and muscle may contribute, the rate of force development in the knee extensors tends to be highly variable, and is mainly driven by inter-individual variability in neural factors (Folland *et al.*, 2014). As our participants were previously untrained, variable increases in neuromuscular activation following our intervention may have outweighed any effect of muscle-tendon unit stiffening. Our training intervention focused exclusively on high intensity RE, and thus

participants were able to increase jump performance through increased force production primarily, without augmenting skill components.

***Limitations***

Our data were collected during a period of re-opening following prolonged national lockdown due to the COVID-19 pandemic. Therefore, we recruited participants from a previously untrained, yet active population, and it would not have been possible to recruit resistance trained men given the prolonged closure of gyms and leisure facilities. As a result, caution should be exercised when extrapolating these results to athletic populations. Moreover, while this is the first study to examine the combined effects of RT with collagen supplementation in middle-aged adults, there is reason to believe that middle-aged women may not respond similarly to men (Magnusson *et al.*, 2007b), so further work is necessary to investigate the combined effects of collagen supplementation and RT on tendon properties in middle-aged women. Finally, we acknowledge the limitations in our method of muscle size assessment (a single 38 mm wide sagittal view of muscle thickness) limits our ability to comment on regional muscle hypertrophy, although it should be emphasized that assessing the effects of RT and collagen supplementation on muscle size was not the primary purpose of this study.

***Conclusion***

This study demonstrates that 12 weeks' high intensity resistance training with 30 g hydrolysed collagen supplementation augments patellar tendon CSA, stiffness, and Young's modulus more than resistance training alone. These findings have implications for exercise, nutrition, and rehabilitation prescriptions in healthy middle-

aged men. Further research is required to elucidate if these effects can be replicated in *middle-aged* women.

# Chapter Eight

# Synthesis and General Discussion

# 8.1 Synthesis

The purpose of this chapter is to synthesise the PhD project with respect to: i. achievement of the original aims and objectives outlined in Chapter One; ii. interpretation of the main findings within the context of mechanistic and practical applications arising from the effects HC with RE in middle-aged men and women; iii. discuss the limitations of the experimental chapters; and iv. provide recommendations for future research.

# 8.2 Achievement of aims and objectives

The aims of this thesis were (i) to investigate habitual collagen intake at population level; (ii) to examine the acute effects of high intensity RE with HC supplementation on markers of collagen turnover in middle-aged men and women; and (iii) to explore the effects of chronic (8 – 12 weeks), high intensity RT with HC supplementation on muscle-tendon properties in middle-aged men and women.

These aims were achieved through completion of the following objectives:

1. To comprehensively quantify the habitual dietary collagen intake of Irish adults and identify population-specific patterns of collagen intake i.e., differences in intake by age and sex. This was achieved through secondary analysis of the most recent National Irish Nutrition Survey in Chapter Three.

2. To determine the optimal dose of HC required to elicit the highest collagen synthesis response following an acute bout of RE in resistance trained, middle-

aged men. This was achieved through the completion of a randomised crossover trial in Chapter Four.

3. To identify the effect of RE with and without 30 g HC on markers of collagen turnover during the late follicular phase of the menstrual cycle in resistance trained, middle-aged, premenopausal women. This was achieved through completion of a case study in Chapter Five.

4. To determine the effect of 8 weeks' field hockey training with high intensity eccentric RT and 30 g HC supplementation on changes in muscle-tendon properties of middle-aged female athletes. This was achieved through a randomised placebo control trial in Chapter Six.

5. To establish the effect of 12 weeks' high intensity resistance training with 30 g HC supplementation on changes in muscle-tendon properties in recreationally active, middle-aged men. This was achieved through a randomised placebo control trial in Chapter Seven.

## 8.3 General discussion

The body of research investigating the effects of collagen ingestion on collagen-rich connective tissue quality, such as skin, muscle, bone, and tendon has grown substantially alongside the completion of this thesis. Studies that have found positive effects of collagen ingestion have used a range of doses (5 – 35 g), typically in the form of 1-2 boluses of a HC or gelatine beverage (de Miranda *et al.*, 2021; Khatri *et al.*, 2021; Holwerda & van Loon, 2022; Bischof *et al.*, 2024). Given this wide range,

it is clear that collagen supplementation protocols vary widely across studies, highlighting the need for clarity on optimal dosing strategies for specific populations and outcomes.

Although the overarching aim of this PhD was to examine the mechano-sensitivity of middle-aged human tendon to RT and HC ingestion, the findings from our secondary analysis of the National Adult Nutrition Survey (NANS) in Ireland (Chapter Three) suggest that habitual dietary collagen intake is insufficient to support optimal collagen turnover or connective tissue health without supplementation. Women and older adults exhibited the lowest levels of dietary collagen intake (~2–3 $g \cdot day^{-1}$), while (young) males consumed slightly more, at ~4 $g \cdot day^{-1}$. This is far below the 5–35 g range commonly used in intervention studies, suggesting that meaningful improvements in tendon and connective tissue health likely require exogenous supplementation and attempts to obtain these quantities through regular diet could be prohibitive. Interestingly, the populations considered most at risk of musculoskeletal injury (females and older adults) (Clayton & Court-Brown, 2008a; Ganse *et al.*, 2014; Peat *et al.*, 2014; Augustsson & Ageberg, 2017) had the lowest intakes. Accordingly, this may indicate that both ageing and female populations are where supplementation may have the most profound effect, thus contributing to the decision to investigate middle-aged males and females in the subsequent experimental chapters.

In Chapter Four, it was demonstrated that RE alone did not increase a systemic biomarker of collagen synthesis (serum PINP concentration) in resistance trained middle-aged men. However, ingestion of 15 g HC prior to RE resulted in an increased serum PINP concentration × time AUC, and 30 g HC further augmented this effect. The HC dose-PINP response was consistent with the AUC of glycine, proline, and hydroxyproline following ingestion 0 g < 15 g < 30 g HC before performing RE. Prior

to the investigation in Chapter Four, two studies had investigated the dose-response of collagen ingestion with a single bout exercise on serum PINP, both of which were performed in young men (Shaw *et al.*, 2017; Lee *et al.*, 2023c). Only one of these studies employed high intensity RE (back squats), and was thus likely to have targeted the muscle-tendon unit (MTU) more effectively (Lee *et al.*, 2023c) compared to high-impact exercise like skipping, which likely stimulates bone more than MTU collagen turnover (Hart *et al.*, 2017). The study by Lee *et al.* (2023c) showed that RE alone increased post-exercise collagen synthesis and this was augmented by 30 g but not 15 g HC ingestion (Lee *et al.*, 2023c). In contrast, one study showed that lower intensity RE may be insufficient to increase serum PINP with or without 30 g HC in young men and women (Aussieker *et al.*, 2023). However, numerous limitations with this study design, e.g. the between group design may have increased between intervention variability, thus potentially confounding an effect of HC, compared to the within-group crossover design used by (Lee *et al.*, 2023c). Furthermore, the use of mixed-sex cohorts rather than solely males in the study by (Lee *et al.*, 2023c) may have masked an effect of HC, as oestrogen is known to affect skeletal muscle and tendon collagen synthesis in females. Finally, vitamin C, an essential co-factor in collagen synthesis, was not added to the supplements in the study by Aussieker *et al.* (2023) but it was in the study by Lee *et al.* (2023c). Chapter Four used a very similar study design to that of Lee *et al.* (2023c), i.e. crossover design, 4 sets of 10-RM, thus it was striking that the high intensity leg press exercise alone was not sufficient stimulus to increase collagen synthesis in middle-aged men, as RE alone had stimulated a collagen synthesis response in young men (Lee et al., 2023b). Accordingly, the findings of Chapter Four suggest that anabolic resistance to connective tissue collagen synthesis in response to RE begins in middle-age, even in resistance-trained men, but this can

be overcome by ingesting increasing doses of HC, at least up to 30 g. Moreover, it is most likely that the measure of type I collagen synthesis measured in Chapter Four is arising from passive connective tissue e.g., tendon and not skeletal muscle, as tendon comprises ~80% collagen, whereas muscle comprises <5% collagen (Kjaer, 2004; Gillies & Lieber, 2011).

Considering that 30 g HC was the highest efficacious dose observed in Chapter Four, it was suspected that such a dose of collagen supplementation would also support collagen synthesis in women. Only one previous well controlled case study examined this effect, and found peak serum PINP concentration and the concentration × time AUC response to RE was higher during the onset of menses (low circulating oestrogen concentration) than during the late follicular phase (high circulating oestrogen concentration). Despite high levels of circulating oestrogen being associated with lower collagen synthesis, 30 g HC ingestion augmented the PINP response (Lee *et al.*, 2024c). The findings of Chapter Five provide further understanding of the collagen synthesis response to RE but this time in the context of naturally menstruating, pre-menopausal, resistance-trained, *middle-aged* women. Two participants completed 4 sets of 10-RM leg press during the late follicular phase of their respective menstrual cycles. The findings suggest that (similar to middle-aged men but unlike young women) whole body collagen synthesis was unaffected by RE alone, but a high dose (30 g) of HC increased the PINP concentration × time AUC, even during the late follicular phase of the menstrual cycle, even when circulating oestrogen concentration is expected to be high. Some evidence suggests that synthetic female sex hormones may inhibit exercise-induced tendon collagen synthesis, potentially through suppression of systemic and local insulin-like growth factor I (IGF-I) (Hansen *et al.*, 2013b). While this evidence is based on oral contraceptive (OCP) users and therefore

not directly applicable to the naturally menstruating middle-aged participants in Chapter Five, it nonetheless supports the broader notion that female sex hormones can modulate collagen responses to exercise. In OCP users, the reduced responsiveness may reflect a direct inhibitory effect of synthetic hormones, or an indirect effect mediated by hormonal changes introduced by OCP use, such as reduced IGF-I levels. Additionally, findings from engineered ligament models indicate that oestrogen may not significantly affect collagen content, but can inhibit lysyl oxidase activity thus reducing collagen cross-linking (Lee *et al.*, 2015).Considering that the participant in Chapter Five experienced no change in serum PINP concentration from baseline following RE, unlike the diminished increase in serum PINP observed in the younger participant in Lee *et al.* (2024c), it is more likely that connective tissue anabolic resistance due to aging occurs similarly in middle-aged women as it does in the male participants, described in Chapter Four.

The findings from Chapter Three suggest a low baseline of dietary collagen intake in middle-aged adults. Following this, the findings from Chapters Four and Five demonstrate that ingestion of 30 g HC with RE is an effective strategy to acutely cause a positive net collagen protein balance (elevated collagen synthesis with decreased collagen breakdown). This suggests that long term, repeated exposure, i.e. RT with chronic 30 g HC supplementation, may positively influence musculoskeletal tissue remodelling in this population. Accordingly, Chapters Six and Seven sought to investigate these effects in middle-aged men and women over 8-12 weeks. Prior to this thesis, four studies have examined HC with RT in young adults on tendon properties (Balshaw *et al.*, 2023; Jerger *et al.*, 2023; Lee *et al.*, 2023a; Lee *et al.*, 2024a).

Two of these studies were conducted on young female soccer athletes, and found that 30 g HC augmented gains in PT stiffness and Young's modulus but not gains in tendon

size following 10 weeks' training that included soccer training and low frequency or low intensity RT (Lee *et al.*, 2023a; Lee *et al.*, 2024a). These studies also measured changes in maximal force production, finding no effect of collagen. However, explosive force is more likely to be influenced by the changes in MTU stiffness (Bojsen-Møller *et al.*, 2005; Quinlan *et al.*, 2017) but this was not assessed. In Chapter Six, by comparison, 22 elite female Master (international women's hockey) athletes completed eccentric-focussed RT three times per week for eight weeks (in addition to their normal hockey training), which was supplemented with either 30 g HC or 30 g maltodextrin with 500 mg vitamin C. The collagen group experienced greater gains in PT CSA, which were most pronounced in the distal portion (+5% for collagen vs. +3.5% for placebo). These are similar to the 4 – 7 % regional PT hypertrophy experienced after 9 – 12 weeks RT in young men (Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009). The higher mechanical strain induced by eccentric RT likely explains these CSA increases, which were not observed in the studies by Lee *et al.* (2023c); (2024c), The greater gains in tendon size in the collagen group of Chapter Six are directly supported by the findings of Chapter Five, where 30 g HC ingested prior to RE in a middle-aged female athlete was associated with a larger serum PINP concentration × time AUC than when 0 g HC was ingested.

It should be noted that it was not possible to measure tendon stiffness in Chapter Six, as the limited access to elite athletes necessitated the set-up of a mobile laboratory at the training camp where they performed their training one day a week for 10 weeks. However, rate of force development (RFD), which is thought to be influenced by MTU stiffness (Bojsen-Møller *et al.*, 2005; Quinlan *et al.*, 2017), increased more in the HC than the placebo group (+27.3% vs. +8%), mirroring the tendon CSA changes in the two groups. These results suggest that gains in PT stiffness in the studies by (2023c);

Lee *et al.* (2024c) likely arose from changes in tendon material properties such as increased collagen fibril density and cross-linking (Heinemeier & Kjaer, 2011; Couppé *et al.*, 2021). Conversely, in Chapter Six, the combined effects of CSA hypertrophy and potential material property changes likely enhanced stiffness, as a larger CSA distributes mechanical stress more effectively (Wiesinger *et al.*, 2016).

In Chapter Seven, 20 recreationally active, middle-aged men completed 12 weeks of RT, training twice per week and supplementing immediately post-exercise with either 30 g HC or maltodextrin (placebo), each supplement enriched with 50 mg vitamin C. RT increased patellar tendon (PT) stiffness and Young's modulus by ~18% without changes in PT cross sectional area (CSA). However, RT with collagen ingestion increased PT CSA at the proximal and distal ends by ~6% and concomitantly increased PT stiffness and Young's modulus by ~50%, which was greater than training alone. Two studies (which both appear to be from the same intervention) found that daily supplementation with HC RT enhanced PT and Achilles tendon hypertrophy but did not augment gains in tendon stiffness or Young's modulus (Jerger *et al.*, 2022; Jerger *et al.*, 2023). This result is unusual, as increased CSA typically contributes to enhanced stiffness (Wiesinger *et al.*, 2016). One possible explanation is that the young participants in the studies by Jerger and colleagues may have already maximised their rate of tendon adaptation through training alone within the study timeframe, leaving limited scope for further augmentation through HC supplementation. Notably, both groups in the studies by Jerger *et al.* (2022); (2023) exhibited substantial tendon hypertrophy, which contrasts with the more modest hypertrophy reported in the literature, including the findings from Chapter Seven.

In Chapter Seven, the absence of PT hypertrophy in the placebo group aligns with findings from Chapter Four regarding the lack of serum PINP increase following RE

alone, suggesting connective tissue anabolic resistance to RE in middle-aged men. Specifically, Chapter Four showed a lack of collagen turnover following resistance exercise alone, indicating that RT may not sufficiently stimulate tendon hypertrophy in this population without additional nutritional support in the form of HC. This also suggests that the increase in serum PINP concentration following 15-30 g HC ingestion largely reflects increases in tendon rather than other connective tissue collagen synthesis.

Although muscle strength, rate of force development (RFD), and muscle thickness, and jump performance increased in both groups, there were no differences between groups in any of these measures. This contrasts with findings from Chapter Six, where elite female Master athletes ingesting HC alongside each RT session experienced greater gains in RFD and tendon size. The difference is likely attributable to the training status of the participants. The men in Chapter Seven were recreationally active but RT-naïve, meaning neural adaptations such as improved motor unit recruitment and firing rates (Folland *et al.*, 2014) likely dominated the RFD improvements in both groups. These neural gains may have masked any additional contribution of increased tendon stiffness in the collagen group. In contrast, the female athletes in Chapter Six were elite international athletes, who had likely already experienced substantial neural adaptations through lifelong training. For this population, material and structural changes in the tendon (e.g., increased stiffness, modulus and CSA) were likely more readily translated into improved RFD, highlighting the importance of considering baseline athlete status when evaluating the functional outcomes of tendon adaptations.

## 8.4 Project limitations and recommendations for future research

*Suggestions arising from Chapter Three*

The dietary data presented in Chapter Three are derived from the most recent National Irish Nutrition Survey, collected from 2009-2011, eight years prior to the start of this PhD project. Consequently the recent surge in the global collagen market may not be reflected in these data (GrandViewResearch, 2024). Moreover, athletic populations are likely underrepresented, as most of the sample reported low levels of physical activity. Future studies should look to employ our system of applying collagen values to food database items within large scale habitual dietary intake surveys to quantify collagen intake in other populations outside of Ireland.

*Suggestions arising from Chapter Four*

Chapters Four and Five measured serum PINP and plasma β-CTX to indirectly assess type I collagen synthesis and degradation, respectively. These biomarkers are traditionally associated with bone turnover (Dolan *et al.*, 2022; Dolan *et al.*, 2024). Future studies could aim to parse the individual soft tissue response by harvesting tissue biopsy from tendon. This approach would allow direct quantification of collagen synthesis by measuring the collagen fractional synthetic rate in the tendon, following infusion of labelled amino acid tracer or doubly labelled water. However, while muscle biopsy is relatively common, a limited number of laboratories perform the more invasive tendon biopsy technique due to technical challenges and limited facilities/expertise. All data collection for this study was performed at South East

Technological University in Carlow, Ireland, which did not have the facilities for muscle or tendon biopsy collection or analysis.

*Suggestions arising from Chapter Five*

In Chapter Five, RE alone did not increase collagen synthesis in a middle-aged, naturally menstruating, resistance trained woman. However, the ingestion of 30 g HC ingestion increased collagen synthesis post-RE in another similar participant. This was a case study involving only two participants, and therefore not statistically powered to detect between group differences, and the findings should be considered exploratory. The recruitment of a larger sample for this study was limited by external factors. Initially, 10 women volunteered between November 2019 and January 2020, and tracked their menstrual cycles for two consecutive months. Due to the COVID-19 national lockdown in March 2020, only two participants had completed a portion of data collection, and the remainder were lost during laboratory closures. Although the remaining eight volunteers self-identified as pre-menopausal, three reported menstrual irregularities which may indicate peri-menopause, early menopause or other endocrine concerns, which emphasises the challenge in categorising hormonal profiles in this demographic (Brambilla *et al.*, 1994; Butler & Santoro, 2011).

Initially, it was intended to confirm menstrual cycle phase using hormone assays, however this was not possible due to the limited number of samples, and this should be included in future work. Additionally, postmenopausal women, including those using exogenous hormone therapy, were not included in this study, which limits the generalisability of these findings to all middle-aged women. Future research should investigate the collagen turnover response to HC and RE across various hormonal profiles in middle-aged women. This could be achieved by utilising a within group

design replication of Chapter Four with stringent subgroup inclusion criteria and data collection at multiple (hormonally divergent) phases of the menstrual cycle, or a between groups design comparing exogenous hormone users with non-users.

*Suggestions arising from Chapter Six*

In Chapter Six, participants were recruited from two international Masters hockey squads (over 35 years-old, and over 40 years-old) who trained together during pre-competition camp. There were several logistical constraints that necessitated adaptation of traditional laboratory methods, and the development of a mobile laboratory. The elite athletes in this study had limited availability, and any data collection or training/nutrition intervention needed to be integrated into their existing programme. Accordingly, pre-and post-intervention testing was required to be completed on specific days before and after the eight-week intervention, meaning all participants were not tested on the same day of their respective menstrual cycles. While this was not expected to have influenced the primary outcome measures used in this study i.e., patellar tendon properties, isometric strength, countermovement jump performance (Burgess *et al.*, 2009; Kubo *et al.*, 2009b; Hansen *et al.*, 2013b; Blagrove *et al.*, 2020), the influence of fluctuating ovarian sex hormones cannot be ruled out entirely. Furthermore, while the participants in this study were naturally menstruating Master athletes, two were current users of combined oestrogen and progesterone oral contraceptive pills (Marviol® 150/30 and Microlite® 100/20). To minimise any influence on between group effects, these participants were divided between the collagen and placebo groups.

Due to logistical challenges (i.e. it was not possible to transport the elite athletes to the university laboratory), and the limitations of the mobile laboratory (i.e. the

ultrasonography could not be synchronised with isokinetic dynamometry or surface EMG), direct measures of tendon stiffness (as performed in Chapter Seven) were not possible. Although this suggests researchers should attempt to recruit from teams of athletes in proximity to their laboratory, it would limit the opportunity of recruiting from truly elite athletes (such as those in Chapter Six), based considerable distances from the University laboratory. Additionally, the athletes in Chapter Six were based across the United Kingdom and Ireland, and only trained together centrally once per week, thus two of the three weekly RT sessions were unsupervised. Every effort was made to ensure compliance, and to standardise volume load across all participants, however drop-out rate remained high, and athletes who reported non-compliance (n = 4) or injury outside the study (n = 3) were excluded.

*Suggestions arising from Chapter Seven*

The participants in Chapter Seven were not resistance trained prior to participation; therefore, the findings may not be directly applicable to more highly trained male middle-aged populations. Both Chapters Six and Seven exclusively investigated middle-aged populations, addressing a significant research gap in an under researched population. Older adults exhibited the lowest collagen intake in Chapter Three, making them a critical population for further investigation. Considering the findings of all four experimental studies in this PhD thesis, future research should prioritise examining the effects of RT with collagen supplementation on muscle-tendon unit properties in older adults (e.g. >65 years-old).

# 8.5 Perspectives

The findings of this thesis have made substantial, novel contributions to our understanding of the tendinous adaptations to resistance training with collagen supplementation. As such, based on currently available data, an updated summary of expected outcomes following acute and chronic interventions are displayed in **Table 1.**

**Table 1.** Summary of expected acute and chronic outcomes following resistance exercise with hydrolysed collagen supplementation in young and middle-aged populations.

| **Population** | **0–6 Hours** | **8–15+ Weeks** |
|---|---|---|
| **Young men** | RE alone ↑ PINP, ↓ β-CTX; 30 g (but not 15 g) HC further augments PINP | RE alone ↑ tendon CSA, stiffness, Young's modulus; HC ↑ tendon CSA; effect of HC on stiffness/modulus/RFD unclear |
| **Middle-aged men** | RE alone: no change in PINP, ↓ β-CTX; PINP ↑ with 15 g HC, further augmented with 30 g HC | RE alone ↑ tendon stiffness and Young's modulus but not CSA; HC augments tendon CSA and further increases stiffness/modulus; HC does not augment RFD but may depend on training status |
| **Young, naturally menstruating women** | Lower collagen synthesis than men; potentially phase-dependent PINP. HC may augment PINP response to RE during low oestrogen phases | ↑ Tendon CSA and stiffness with RE alone; enhanced by 30 g HC. Limited long-term data on RFD, effects unclear with HC |
| **Young OCP users** | No change in PINP after RE. No acute data with HC ingestion | ↑ Tendon CSA and stiffness with RE + 30 g HC; Limited long-term data on RFD, effects unclear with HC |

| | | |
|---|---|---|
| **Middle-aged, naturally menstruating women** | Attenuated PINP response to RE; rescued by 30 g HC. Data very limited | ↑ Tendon CSA with RE alone, augmented with HC; stiffness change unknown. ↑ RFD with RE, augmented by HC |

*RE*, Resistance Exercise; *PINP*, procollagen type I N-propeptide; β-*CTX*, C-terminal telopeptide of type I collagen; *HC*, Hydrolysed Collagen; *RFD*, rate of force development; *OCP*, oral contraceptive pill

One of the key strengths of this thesis is the use of both acute response and chronic training interventions using collagen supplementation with resistance training. This means that the findings are directly applicable to applied practice in strength and conditioning and sports nutrition and do not rely on extrapolation from short-term studies. If middle-aged individuals have determined through needs analysis, or similar, that tendon adaptation and explosive exercise performance is a performance priority, the data presented in this PhD thesis provide a relatively high degree of certainty that hydrolysed collagen ingestion, when timed appropriately around high intensity resistance exercise, can support this goal.

Despite the beneficial effects of collagen supplementation described in this thesis, it is important to emphasise that these are context specific. Collagen was consumed around resistance exercise, which appears to be a prerequisite for the beneficial effects observed. The type of exercise is of high importance, for example protocols that impose high cyclical strain on the muscle–tendon unit are likely to remain the most potent stimuli for collagen turnover and tissue adaptation. It is therefore unlikely that increasing dietary collagen intake in the absence of such loading would elicit positive effects on tendon structure. As such, more global recommendations for middle-aged adults should still focus on physical activity itself i.e., encouraging high and sustained levels of activity across the lifespan.

Throughout this thesis, it has also been speculated that the positive adaptions in tendon (increased size, stiffness and elastic modulus) may contribute to a reduction in injury risk. This leans on the strong positive relationship between tendon stiffness and ultimate loading capacity demonstrated across various species (LaCroix *et al.*, 2013), and the suggestion that increases in size and stiffness may reduce strain during loading. (Mersmann *et al.*, 2023) suggest that imbalances in muscle and tendon adaptation may lead to excessive strain on tendons during loading tasks. Tendons that are too compliant relative to the muscle's force-generating capacity may be more susceptible to injury. In this context, the increased tendon size and stiffness observed in this thesis are likely to reduce strain for a given force, and therefore enhance safety and resilience under load. This physiological and biomechanical rationale underpins the application of high intensity training and collagen supplementation in this context, but requires prospective studies linking changes in morphological and material properties to injury incidence.

We observed improvements in muscle size, as well as maximal and explosive strength, following medium-to-long-term high-intensity resistance training regardless of supplementation. Importantly, these findings suggest that tendon adaptations can be enhanced through collagen supplementation without compromising muscle hypertrophy or requiring major changes to training or nutrition. This supports the practical use of collagen for individuals already engaged in structured training, as a simple, low-burden strategy to support tendon adaptation, particularly during phases when tendon adaptation, injury resilience, or explosive strength are a priority.

The timing of collagen supplementation remains a relevant practical consideration. The studies investigating collagen supplementation on postprandial aminoacidemia and collagen turnover, including those in this thesis, are tightly controlled laboratory

protocols, with collagen ingested in a fasted state, usually as a liquid. However, in applied settings, active adults and athletes may choose to consume collagen in other forms e.g., gelatine-based confectionary or alongside pre-training meals containing carbohydrate, fat, protein or fibre. The inclusion of mixed nutrients is likely to delay gastric emptying and subsequent digestion and absorption of the collagen supplement, which may affect the timing and magnitude of amino acid appearance in the bloodstream. Whether this has a meaningful impact on collagen synthesis or tissue adaptation is currently unknown and represents an important avenue for future research.

We used the patellar tendon as our model due to its key role in transmitting large forces during locomotion and explosive athletic movements (e.g., sprinting, jumping, changing direction), as well as its superficial anatomical location and accessibility for repeated assessment. In this context, increased cross-sectional area and stiffness are interpreted as beneficial adaptations. However, whether these same effects are desirable across all tendons is less clear. For example, in tendons such as those of the rotator cuff, excessive increases in tendon cross-sectional area might not be beneficial or have unintended consequences. Due to the confined anatomical space of the shoulder joint and the role of tendon glide in normal movement, tendon hypertrophy in this region could theoretically increase the risk of impingement or disrupt joint mechanics. This highlights the need to consider tendon location, function, and surrounding anatomy when generalising findings.

It is also worth noting that while no adverse effects of collagen supplementation were observed in the studies presented in this thesis or in the wider literature. Therefore, it appears the interventions described in this thesis are safe and well tolerated in active individuals, however, this may not extend to all populations. For example, in

conditions characterised by pathological collagen accumulation such as systemic sclerosis or organ fibrosis, collagen supplementation may be contraindicated due to the potential risk of exacerbating fibrotic processes. Although speculative in the context of the current work, it highlights the need for caution when considering collagen interventions outside of healthy, active cohorts.

Additionally, factors such as obesity and chronic low-grade inflammation are known to impair collagen synthesis and alter extracellular matrix remodelling. These conditions may disrupt the normal balance between synthesis and degradation, potentially blunting the effectiveness of collagen-targeted interventions. While these issues fall outside the scope of this thesis, they may influence the efficacy or generalisability of the findings to broader or more clinical populations and should be considered in future research.

## 8.5 Conclusion

The first finding of this thesis, based on exploratory analysis, is the first comprehensive evidence of low dietary collagen intake at population level, which is lowest in women and older adults (Chapter Three). These findings highlighted a nutritional gap in habitual diets that may benefit from supplementation, with those groups reporting the lowest intakes likely to experience the greatest benefit. Prior to this thesis, a relatively small number of researchers had investigated the collagen synthetic response to RE with collagen supplementation in young adults. However, Chapter Four is the first to demonstrate that RE alone does not stimulate collagen synthesis in middle-aged, resistance trained men, but that HC ingestion recovers this response in a dose-dependent manner, where 15 g was beneficial, but 30 g was the

most efficacious dose. Furthermore, Chapter Five suggests that premenopausal, middle-aged, resistance trained women may experience similar connective tissue anabolic resistance to men, but this too can also be rescued by ingesting 30 g HC prior to high intensity RE (even during the late follicular phase, when circulating oestrogen concentration is high). Accordingly, these findings suggest that 30 g HC with chronic RT could impact tendon properties in middle-aged women and men. In Chapter Six, elite female (international Master) athletes completed eight-weeks' (three times weekly) eccentric RT, integrated into a pre-competition hockey training camp. These athletes experienced greater increases in tendon size and multi-joint rate of force development when they were supplemented with 30 g HC. Not only is Chapter Six the first study to report benefits of HC with RT in middle-aged female athletes, but it is also the first to demonstrate that HC supplementation can influence explosive exercise performance by enhancing tendon properties. In Chapter Seven, recreationally active but previously untrained middle-aged men took part in a 12-week high intensity RT intervention, which led to increases in tendon stiffness, Young's modulus, and lower limb rate of force development regardless of supplementation. Only RT with 30 g HC ingestion led to tendon hypertrophy, which was not seen in men who performed RT alone. Furthermore, 30 g HC ingestion enhanced gains in tendon stiffness, but this did not lead to enhanced explosive exercise performance in middle-aged men. Chapters Six and Seven clearly demonstrate long-term collagen supplementation with RT positively impacts tendon properties of middle-aged adults and suggests that such an intervention can improve tendon health and athletic performance in this population, yet subtle differences in training adaptation can be expected due to sex, athletic status, and training history.

# Chapter Nine

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# 9.1 References


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# Chapter Ten

# Appendices

# Appendix I – Participant Information Sheet and Informed Consent for Chapters Four and Five

**Participant Information Sheet**

**LJMU's Research Ethics Committee Approval Reference: 19/SPS/049**

**YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET**

**Title of Study:**

The dose-response of vitamin C-enriched collagen supplementation on markers of collagen synthesis after exercise in healthy middle-aged men and women following resistance exercise

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You are being invited to take part in a research study. Before you decide it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you want to take part or not.

**1. What is the purpose of the study?**

To investigate the optimal dose of collagen and vitamin C supplementation for maximal muscle and tendon collagen synthesis in middle-aged men and women.

The optimal dose of collagen for maximum collagen synthesis might differ between men and women. As people age, a blunted response to anabolic stimuli such as exercise and nutrition have been observed. Therefore, it is possible that higher doses of collagen ingestion in combination with resistance exercise may be required to stimulate tendon collagen synthesis for middle-aged adults. Females in particular may display different responses to training and collagen ingestion as estrogen levels have been shown to impact collagen synthesis. Women over the age of 40 usually display lower estrogen levels compared to younger women, and are less likely to be controlling their hormones through oral contraceptive use. Postmenopausal women have markedly low levels of estrogen, which can be as low as the levels observed in men. This shows the importance of studying the impact of different dosages of collagen in the middle aged population, as it is unlikely to be a one size fits all recommendation. Maintaining tendon health throughout the lifespan is important for joint function, athletic performance and quality of life.

Middle aged men and women are at risk of sustaining injuries in collagen-rich tissues, such as ligaments and tendons. Ageing is associated with a reduction in both the stiffness and strength of human tendons (such as the Achilles or patellar tendons). Having strong and stiff tendons is dependent on the collagen content and the amount of crosslinks within the collagen. A smaller and more compliant tendon reduced the amount and speed of force transferred during movement, which can affect quality of life, increase rates of trips/falls in older adults, and increase injury rate in active people. A larger, stiffer tendon can increase strength, power, and speed.

A previous study has demonstrated that collagen supplementation, in combination with exercise, increases collagen synthesis in young adults. However, this has not been examined in other active populations. The purpose of this study is to determine how much of a collagen supplement is required to result greater collagen production after resistance exercise in middle-aged men and women. By understanding this, we aim to make nutritional and exercise guidelines for middle aged adults to optimize tendon health and/or athletic function.

## 2. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form – if applicable. You can withdraw at any time by informing the investigators without giving a reason and without it affecting your rights.

## 3. May I be excluded from the study?

Yes. You can be excluded from the study at any time point if you do not adhere to the study conditions

## 4. Why have I been invited to participate?

**You are likely to be eligible for this study if you fulfil the following criteria.**

- Be healthy male or female:
  - Age 40-65 years
  - No history of patellar tendon injuries
  - No history of lower limb musculoskeletal injuries in the past 12 months
  - Currently performing a structured exercise training programme (including the lower limbs) (at least 3 days per week)
  - Non-smokers (including e-cigarettes)

**You MUST NOT take part in this study if:**

- You are younger than 40 or older than 65 years
- Female participants should not be using Oral Contraceptives or receiving Hormone

Replacement Therapy

- Vegans (collagen is derived from mammals and fish)

Smokers (including e-cigarettes)

## 5. What will happen to me if I take part?

You will be required to visit the laboratories located in Carlow IT for four times over four weeks (once per week).

After Familiarization and three experimental tests on Visit1, you will be asked to perform four sets of ten repetitions on a leg press and ingest different doses of collagen (0 g, 15 g, and 30 g) with vitamin C (50 mg) during three visits for men, and four visits for women.

***24 hours before visiting the laboratory for four times, you will be asked to fast 12-hour overnight except for water and refrain from strenuous physical exercises, consumption of alcohol and caffeine.***

**Visit 1 (100 minutes)**

**Familiarization session, 10-RM test and assessment of tendon properties**

1) Tendon properties assessment (30 minutes)

You will be seated on an isokinetic dynamometer (specialized equipment recording muscle force around a joint) and the knee joint will be set at 90 ° knee flexion, in order to measure the length of the patellar tendon (just below the knee cap) by using an ultrasound probe. The probe will then be placed on the skin over the points associated with 25, 50 and 75% tendon length, and the scans will be recorded to measure tendon cross sectional area at these points. During a ramped isometric maximum voluntary contraction (MVC, which is a contraction with a gradual increase in force to a full MVC) the ultrasound probe will be placed longitudinally over the tendon and the tendon elongation will be recorded. These measurements will subsequently be used to assess differences in tendon size and stiffness. All measurements will be performed on the right leg of the participants.

Familiarization and 10 RM Leg Press (40 – 50 minutes)

A sport scientist will demonstrate and instruct proper leg press technique to the participants. In order to prescribe individualized training load for the main testing session, you will perform 10-repetition maximum (10 RM) test. 10 RM load will be determined within four trials with rest periods of 3 - 5 minutes. You will start with a warm-up session consisting of lower limb stretching and performing leg press (5 to 10 repetitions with a light-to-moderate load). An initial weight (50%– 70% of your maximal capacity) will be selected and resistance will be progressively increased by 2.5-20 kg until the point at which you cannot complete 10 repetitions. The final weight lifted successfully with the proper technique will be used as the 10-RM load.

**Filling in a food and drink diary (8 minutes per day for three days)**

In order to maintain similar eating patterns over experimental periods, you will be asked to fill in a drink and food diary for 3 days. You will need to fill in the dairy on Thursday, Friday, and Saturday, which will represent your weekly eating patterns. The diary will be provided during Visit 1 and you will be shown how to complete it correctly.

**Visit 2 – 4/5 (7 hours)**

**Main testing sessions consisting of three different trials**

Participation

1) Resting blood samples

You will be asked to visit the laboratory 1 h before performing two resistance exercises. Upon arrival to the laboratory, 10 ml of resting blood sample will be taken from a superficial forearm vein. It should be noted that 10 ml of blood will be drawn at this time point only and a small volume of blood (5 ml) will be drawn at the rest of time point. Then, you will ingest one of different doses of collagen (0 g, 15 g, and 30 g) with vitamin C (50 mg) in 400 ml of water. Two more 5 ml blood samples will be drawn at 0.5 h and 1 h after consumption of the supplement.

2) Leg Press Exercise

After a warm up, you will perform the lying leg press exercise (4 sets of 10 RM interspersed with 2minute rest periods) and one blood sample will be taken immediately after the exercises.

3) Post-exercise condition

After completion of the leg press, four blood samples will be taken at 1 h-, 2 h-, 4 h-, and 6 h-post exercise. As you will rest in the laboratory for 6 hours, it is suggested that bring a laptop or books to spend the time in the laboratory.

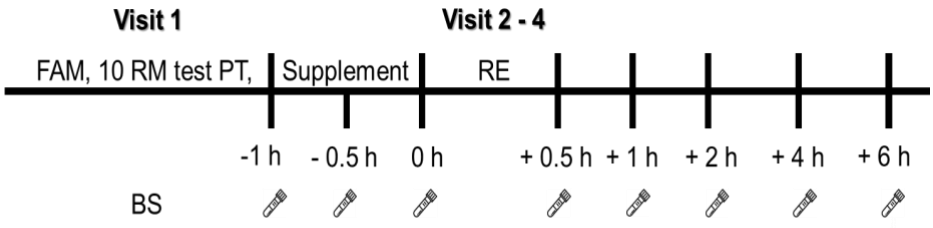



**Figure 1.** Schematic time line of study. RE, resistance exercise; BS, blood sample; FAM, Familiarisation session; 10 RM test, 10 repetition maximum test; PT, patella tendon assessment.

Vitamin C-enriched collagen supplement

Hydrolyzed collagen is produced from collagen found in the bones, skin, and connective tissue of animals, therefore it is an animal produce which is safe for human consumption. A hydrolyzed protein is a protein which has been at least partially broken down into its component amino acids. The specific amino acids contained in collagen protein are glycine (Gly), hydroxyproline (Hyp), proline (Pro) and alanine (Ala).

*Males & Postmenopausal Females*: On three separate occasions, you will be asked to ingest (drink) a different dose of hydrolyzed collagen (either 0 g, 15 g, or 30 g) each mixed with 50 mg of vitamin C in 400 ml of water. This is similar to supplementation with a whey protein shake, which you may be familiar with, which used different dairy proteins. The small amount of vitamin C is added as it is essential for collagen synthesis. Each solution will be mixed with either 30.5 g, 17g or 0 g of the food additive maltodextrin. Maltodextrin (a non-sweet carbohydrate product with a high glycemic index made from starch) to ensure each beverage contains the same number of calories. Lastly, a small amount of non-calorie sweetener will be added for better taste. The total kilocalorie content of each supplement drink is 122 Kcal (3.6 Kcal per 1 g of hydrolyzed collagen and 4 Kcal per 1 g of maltodextrin).

*Pre-menopausal Females*: On four separate occasions, you will be asked to ingest (drink) a different dose of hydrolyzed collagen (either 0 g or 30 g) each mixed with 50 mg of vitamin C in 400 ml of water. You will consume each dose on day 0 (1 day post menstrual cycle) and day 14 (14 days post menstrual cycle). This will result in 4 total visits, 2 on days when your estrogen levels are highest, and 2 on days when your estrogen levels are lowest. This is similar to supplementation with a whey protein shake, which you may be familiar with, which used different dairy proteins. The small amount of vitamin C is added as it is essential for collagen synthesis. Each solution will be mixed with either 30.5 g, 17g or 0 g of the food additive maltodextrin. Maltodextrin (a non-sweet carbohydrate product with a high glycemic index made from starch) to ensure each beverage contains the same number of calories. Lastly, a small amount of non-calorie sweetener will be added for better taste. The total kilocalorie content of each supplement drink is 122 Kcal (3.6 Kcal per 1 g of hydrolyzed collagen and 4 Kcal per 1 g of maltodextrin).

Blood sampling

5 ml of blood will be drawn from a superficial forearm vein at the following time points: at rest, 0.5 h and 1 hour after consumption of the supplement, immediately after the exercise, 1h, 2h, 4h and 6 hpost exercise. A total of eight blood samples will be taken in each visit except for Visit 1. Cannulation, a small plastic tube inserted into the vein to allow for multiple blood sampling and thereby reducing the need for

repeated needle insertions, shall be performed at the start of the day and removed at the end. This is a routine procedure and should result in no more than a small amount of discomfort on insertion of the needle.

**6. Are there any risks/benefits involved?**

**Benefits**

In participating in this study no direct benefit to you will occur but it is hoped that if the optimal dose of collagen will be found, this result will be linked to a future study, which will investigate whether long-term resistance training with collagen supplementation affects muscle and tendon properties compared to resistance training alone. Whilst participating you may experience and learn about ideas around how tendons play an important role during resistance exercise. Also, taking part in this study will help us understand how we can improve athletic performance and health, and reduce injury risk in young and older people, as tendons also respond to exercise and nutrition.

**Possible risks**

If at any point during the protocol you feel uncomfortable or unable to continue, testing will be ceased immediately.

*Risk of muscle pain caused by resistance exercise*

Proper techniques of the resistance exercise will be provided in a 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. As you are already resistance trained and experienced with leg press exercise, we expect you to experience minimal muscle soreness in the days following the resistance exercise.

*Blood sampling*

Blood samples will be taken on several occasions. You will feel a sharp pain when the needle is inserted but this will be short-lived. The researchers are also experienced in this technique so the pain experienced will be minimal. You may also develop a small bruise on your arm, which can be prevented by applying pressure on the arm when the cannula has been taken out (the researcher will remind/instruct you to do this). Also, a rounded band will be attached at the site of blood sampling to prevent any contamination after completion of blood sampling. The potential risks are small and may include the following: infection (a slight risk any time the skin is broken), bleeding of the site, hematoma (blood collected beneath the skin that

usually disappear over a few days but under very rare conditions could require surgery), and bruising of the area.

## 7. What will happen to my tissue samples

As part of this research project, blood samples will be processed immediately after collection. Blood samples will be kept on ice and centrifuged after 60 min. Whole blood samples will not be stored. The serum/plasma shall be stored in tubes and stored at -80°C for later analysis in a secure freezer in the Institute of Technology, Carlow. All samples will be stored anonymously, with all identifiable information (e.g. your name) being removed. Samples will receive a laboratory code, with only those involved in the research having access to who provided the samples. All samples will be analysed to measure as they provide information about markers of collagen synthesis in response to resistance exercise and different doses of collagen with vitamin C. Thus, there will be two serum analyses performed (C-terminal telopeptide of type I collagen (CTX-I) and serum amino acid analysis).

You may withdraw consent for this research study at any time, without any reason, and this does not affect any of your rights. If you withdraw from the study, unless you state otherwise, samples which have been collected whilst you have been in the study will be used for research as detailed in this participant information sheet. You are free to request that your serum/plasma samples are destroyed at any time during and after the study. Your serum/plasma samples may be used for future research. We cannot tell you at this moment in time what this research will entail or what analyses will be carried out but we can assure you that all appropriate legal and ethical approvals will be in place. These procedures are in accordance with the Human Tissue Act 2004 Research Codes of Practice. If you wish to discuss withdrawing consent, please contact the LJMU Human Tissue Coordinator at Human.tissue@ljmu.ac.uk. All processes are fully compliant with the Human Tissue Act 2004.

## 8. What will happen to the data provided and how will my taking part in this project be kept confidential?

The information you provide as part of the study is the study data. Any study data from which you can be identified (e.g. from identifiers such as your name, date of birth, audio recording etc.), is known as personal data. This includes more sensitive categories of personal data (sensitive data) such as your race; ethnic origin; politics; religion; trade union membership; genetics; biometrics (where used for ID purposes); health; sex life; or sexual orientation.

When you agree to take part in a study, we will use your personal data in the ways needed to conduct and analyse the study and if necessary, to verify and defend, when required, the process and outcomes of the study. Personal data will be accessible to the study team only. In addition, responsible members of Liverpool John Moores

University may be given access to personal data for monitoring and/or audit of the study to ensure that the study is complying with applicable regulations.

When we do not need to use personal data, it will be deleted or identifiers will be removed. Personal data does not include data that cannot be identified to an individual (e.g. data collected anonymously or where identifiers have been removed). However, your consent form, questionnaires and contact details will be retained for 5 years.

Personal data collected from you will be recorded using a linked code – the link from the code to your identity will be stored securely and separately from the coded data

You will not be identifiable in any ensuing reports or publications.

With your consent, we would like to store your contact details so that we may contact you about future opportunities to participate in studies.

### 9. What will happen to the results of the research project?

The results of this study are expected to be published in a scientific journal, but names of participants will never be published.

### 10. Who is organising the study?

This study is organised by Liverpool John Moore's University

### 11. Who has reviewed this study?

This study has been reviewed by, and received ethics clearance through, the Liverpool John Moore's University Research Ethics Committee (Reference number: 19/SPS/049).

### 12. What if something goes wrong?

If you have a concern about any aspect of this study, please contact the relevant investigator who will do their best to answer your query. The researcher should acknowledge your concern within 10 working days and give you an indication of how they intend to deal with it. If you wish to make a complaint, please contact the

chair of the Liverpool John Moore's University Research Ethics Committee (researchethics@ljmu.ac.uk) and your communication will be re-directed to an independent person as appropriate.

## 13. Data Protection Notice

There are two sponsors for this study. Liverpool John Moores University is the sponsor for this study based in the United Kingdom while Institute of Technology, Carlow is the sponsor based in the Republic of Ireland. We will be using information from you in order to undertake this study and will act as the data controller for this study. Liverpool John Moores University and Institute of Technology Carlow will keep identifiable information about you for 5 years after the study has finished

As higher education institutes, we use personally-identifiable information to conduct research to improve health, care and services. As a publicly-funded organisation, we have to ensure that it is in the public interest when we use personally-identifiable information from people who have agreed to take part in research. This means that when you agree to take part in a research study, we will use your data in the ways needed to conduct and analyse the research study. Health and care research should serve the public interest, which means that we have to demonstrate that our research serves the interests of society as a whole. We do this by following the UK Policy Framework for Health and Social Care Research.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the study to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information by contacting Tina Sparrow, the Liverpool John Moores University Data Protection Officer at DPO-LJMU@ljmu.ac.uk. Any queries in relation to the GDPR or Data Protection at Institute of Technology Carlow can be addressed to the Institute's Data Protection Oversight Group at gdpr@itcarlow.ie or by accessing https://www.itcarlow.ie/resources/data-protection.htm.

If you wish to raise a complaint on how we have handled your personal data, you can contact our Data Protection Officer who will investigate the matter. If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner's Office (ICO).

**This study has received ethical approval from LJMU's Research Ethics Committee** *(insert REC reference number and date of approval).*

**14. Contact for further information**


| | |
|---|---|
| Christopher Nulty (Lecturer/Postgraduate Researcher) | Dr Rob Erskine (Academic Supervisor) |
| Department of Science & Health Sciences | Research Institute for Sport & Exercise Sciences |
| Institute of Technology Carlow | Liverpool John Moores University |
| Kilkenny Road | Byrom Street |
| Carlow | Liverpool |
| R93 V960 | L3 2AF |
| Tel: +353 (0)59 917 5590 | Tel: +44 (0)151 904 62 |
| E-mail: C.Nulty@2019.ljmu.ac.uk | E-mail: R.M.Erskine@ljmu.ac.uk |


**Thank you for reading this information sheet and for considering to take part in this study.**

*Note: A copy of the participant information sheet should be retained by the participant with a copy of the signed consent form.*

**PIS Date:** ***20/10/18*** **PIS Version No:** ***0.1***

# 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<br>*(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-Cure 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 | |

# Appendix II – Participant Information Sheet and Informed Consent for Chapters Six and Seven

# Participant Information Sheet Chapter Six

**Title of Study**: Does collagen supplementation enhance patellar tendon properties in female hockey players?

**Name and Contact Details of the Principal Investigator:**

Christopher Nulty,

Lecturer in Sport & Exercise Science (Physiology & Nutrition), South East Technological University

PhD candidate, Liverpool John Moores University

E: Christopher.Nulty@setu.ie

T: 083 4526370

**Name and Contact Details of Supervisor:**

Dr. Robert Erskine,

Reader in Sport & Exercise Nutrition

Liverpool John Moores University

E: R.M.Erskine@ljmu.ac.uk

You are being invited to take part in a research study. You do not have to take part if you do not want to. Please read this information, which will help you decide.

## 1. What is this study is about?

I am investigating the effects of collagen supplementation combined with resistance exercise in healthy, middle-aged men and women. It has been shown that this combination of training and supplementation enhances the tendon properties of profession women's soccer and in young men. These adaptations are likely to reduce the risk of soft tissue injury, and enhance performance in explosive actions (such as high-speed running and change of direction). The overall aim of the study is to determine whether or not collagen supplementation improves tendon size, and lower body strength and power to a greater extent than training alone. This will help inform training and nutrition protocols for middle-aged trainees.

## 2. Do I have to take part?

No. It is up to you to decide whether to take part. If you do decide to take part, the next step will be to complete the short screening form attached by email. You can withdraw at any time by informing the investigators without giving a reason.

## 3. May I be excluded from the study?

Yes. You can be excluded from the study at any time point if you do not adhere to the study conditions.

## 4. Why have I been invited to participate?

You are being asked to participate due as you meet the following criteria: You are likely to be eligible for this study if you fulfil the following criteria.

- Healthy participant, Age 35-59 years
- No history of patellar/Achilles tendon injury from the list below:
    - Previous Achilles tendon rupture in the past 12 months
    - Patellar tendon rupture in the past 12 months
    - Knee replacement
    - Diagnosis of osteoporosis
- No history of lower limb musculoskeletal injuries in the past 6 months
- Free from cardiovascular and metabolic diseases
- Physically active

You MUST NOT take part in this study if:

- Over/under requirement age
- Vegan (Collagen is derived from mammals and fish)
- Any contraindication identified in the Physical Activity Readiness Questionnaire (PAR-Q)
- Smokers (including e-cigarettes)

## 5. What will happen to me if I take part?

You will be report to training camp on Sundays as normal. During the first Sunday of camp you will undertake approximately 15 – 20 mins of assessments to measure the following after a brief warm up:

**WEEK 1**

~ 5 mins: Patellar tendon size and quadriceps muscle thickness measured by ultrasound (please wear shorts with no leggings for this assessment). You will remain seated while the investigator takes images on a portable ultrasound device.

~ 5 – 10 mins: Strength and power assessments -

- Isometric mid-thigh pull test (pushing into an immovable bar for 2 – 3 seconds while force output is measured). 1 min rest between attempts.
- Countermovement Jump test (2 – 3 maximal vertical jumps with various power measurements taken, 1 min rest between attempts

- 20 m sprint test (2 – 3 maximal sprints, with splits timed at 5, 10, and 20 metres). 1 min rest between attempts.

**WEEK 2 – 9**

You will report to training as normal on Sundays. Thirty minutes before each session you will be required to drink a orange flavoured beverage provided by the research team. There will then be additional strength exercises added to your warm-up. The strength exercises will consist of 4 sets of 6 – 8 repetitions of squats on an iso-inertial flywheel device (flywheel squats for short):

Fig 1. Example of flywheel squats being performed

You will then proceed with hockey training as normal on that day. During each week you will be asked to perform 2 extra brief training sessions in your own time. The training sessions will last a maximum of 5 – 10 mins and can be performed at home or in the gym. These sessions will involve 3 – 6 sets of various repetitions of rear foot elevated split squats. You will also be provided with sachets of the supplement drink to take home, and consume 30 minutes before each training session. The researcher will provide additional guidance and details on how, and when to perform these additional sessions:

Fig 2. Example of rear foot elevated split squats being performed

**WEEK 10**

On the final day of camp, you will be retested on all of the assessments outlined in day 1

(~ 15 mins per player)

After testing you can proceed with hockey training as normal.

## 6. Complete a food and drink diary.

At the end of Day 1, a food and drink diary will be provided in order to measure your energy and nutrient intake. You will be asked to record the diary for three days (Thursday, Friday and Saturday) at home. Detailed instruction is on the first page of the diary. This will be repeated during week 10 to determine if your dietary habits have changed over the course of the study.

## 7. Nutritional Supplementation

You will be randomly assigned to either the collagen group, whey group or control group. Neither you nor the researchers will be aware of which group you have been assigned to. During the training period, you will be given a supplement to be consumed with each resistance training session (3 times per week). The supplements used in this study will comprise either **30 g hydrolysed collagen** (derived from bovine skin, so vegans, vegetarians and anyone who suffers from a meat allergy will not be able to participate in this study) or **30 g maltodextrin** (a commonly used high-glycemic food additive derived from starch in potato, rice, and corn), with the latter being consumed by the control group. All groups will also be required to consume a chewable 100 mg **vitamin C** tablet at the same time as consuming the beverage. The collagen, whey and maltodextrin doses are low, with the collagen and whey constituting 20-25% of the recommended daily intake of protein and the maltodextrin comprising 10% of the recommended daily amount of carbohydrate in adults. The vitamin C dose is extremely low and come with no known adverse risks or side-effects at such low doses. Although there is no documented evidence of a deleterious effect from the ingestion of collagen, a rare allergy, sensation of unpleasant taste or feeling of heaviness in the stomach might occur. If any of these transpire, please inform the researchers immediately. The low dose of maltodextrin carries minimal risk or adverse effects, although the high-glycaemic index of this food additive can cause an increase in blood sugar levels, **so diabetics should be aware of this**. However, due to the exercise-induced transportation of blood glucose into the muscle, the insulin response to the increase in blood glucose following maltodextrin ingestion is likely to be blunted. Much larger doses of maltodextrin than that used in this study have been associated with bloating, flatulence and in severe cases, diarrhoea. However, this risk is very low with the highest dose used in this study. During the baseline and post-training tests, the supplementation will be given as one-off dose while during the training intervention, the supplementation will be given immediately after each training session (three times per week for 10 weeks).

## 8. Are there any risks/benefits involved?

**Benefits**

You will learn techniques of performing resistance exercises and learn your maximum strength and power. After 10-week resistance training, it is expected that your strength and muscle size will be increased, and your tendon health improved regardless of which supplement group you are in. This will also have knock on effects on your hockey performance, making you more resilient to soft tissue injury, and enhance performance of sprinting, side-cutting, and changing direction.

**Possible risks**

There is a small isk of muscle pain caused by resistance exercise. Proper techniques of the resistance exercises will be provided in a 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 might experience delayed onset muscle soreness (DOMS) (feel pain and stiffness in muscles) for 24 – 72 hours after the first training session. If you have severe DOMS (feel severe pain whenever you move your body), further training sessions will be delayed until you are fully recovered from the DOMS.

## 9. What will happen to the data provided and how will my taking part in this project be kept confidential?

Throughout the study your personal information will be kept entirely confidential. Instead of your name, your data will be given an identification code, so that you will not be identifiable. Your personal data will be destroyed four years after the testing is complete. The results of this study are expected to be published in a scientific journal, but names of participants will not be published. In addition, responsible members of South East Technological University may be given access to personal data for monitoring and/or audit of the study to ensure that the study is complying with applicable regulations.

## 10. What will happen to the results of the research project?

The results of this study will be used for a chapter in a PhD thesis. They are then expected to be presented at a scientific conference, and published in a scientific journal. Names of participants will never be published, nor will individual participants be identifiable from any results.

## 11. Who is organising the study?

This study is organised by South East Technological University, Carlow, and Liverpool John Moores University.

## 12. Who has reviewed this study?

This study has been reviewed by, and received ethics clearance through, the Carlow Research Ethics Committee.

## 13. What if something goes wrong?

If you have a concern about any aspect of this study, please contact the relevant investigator who will do their best to answer your query. The researcher should acknowledge your concern and give you an indication of how they intend to deal with it.

Thank you for reading this information sheet and for considering taking part in this study. Please follow the link to complete the screening form included in the email.

**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.

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***

# Participant Information and Informed Consent Form Chapter Six

## Title of Study: 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.

### 1. 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.

## 2. PROCEDURES

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

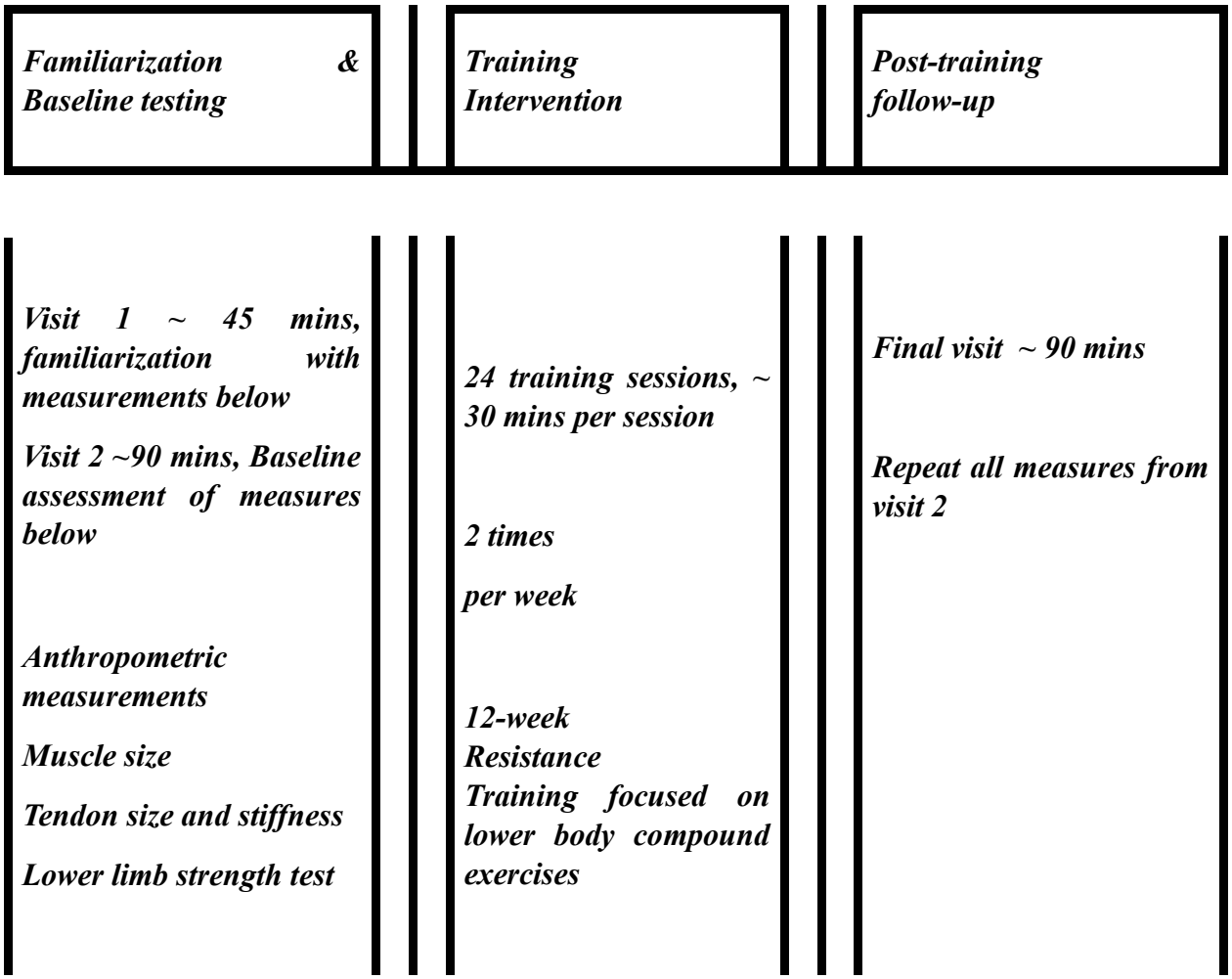



***Figure 1. An overview of the study intervention periods and measurements. For further information please consult the participant information sheet***

## 3. 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.

## 4. 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

## 5. 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)

## 6. 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.

## 7. 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.

## 8. IDENTIFICATION OF INVESTIGATORS

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

Christopher Nulty

Lecturer, Dept. of Science & Health

South East Technological University

Christopher.nulty@setu.ie; c.nulty@2019.ljmu.ac.uk

Dr. Robert M. 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

Kieran Phelan

Postgraduate Researcher

Dept. of Science & Health

Institute of Technology Carlow

Kieran.phelan@itcarlow.ie

## 9. 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***

## Appendix III – Food and Drink Diary


## Research Institute for Sport & Exercise Science

# FOOD AND DRINK DIARY

Participant ID:

# How to complete the food record booklet

1. The purpose of asking you to record your dietary intake is to assess the relationship between habitual diet and body composition.

2. Please record everything you eat and drink each day for three consecutive days (e.g. Thursday, Friday and Saturday). It's important that you do not change your eating habits in any way due to the dietary analysis, so please be as honest as you can and eat the foods that you normally eat.

3. Please give us as much detail as possible about what you eat and drink, i.e. description (e.g. wholemeal or white bread), portion size, packaging, etc., and what time you eat and drink.

4. Please state the method of cooking e.g. boiled, grilled, fried.

5. Please state the amount of food eaten (small, medium and large portion) and drink consumed, e.g. 300 mL mug of tea, ½ or full pint beer, small/large (175/250 mL) glass wine.

6. Please state the brand of food wherever possible, e.g. Heinz Cream of Tomato Soup, McVities Digestive Biscuits.

7. If two items are eaten together, please state the individual amounts, e.g. apple and custard: fist-size helping of stewed apple and half a 350 mL can Ambrosia Custard.

8. For items regularly consumed (e.g. cup of tea), please state the components, (i.e. water, milk, sugar) once. We will then assume all mugs of tea are the same thereafter.

9. For the milk- please also specify skimmed, semi-skimmed, full-fat; and whether cow, soya, goat, rice source

10. For the sugar- please also specify whether brown, white, cane etc.

11. For the tea itself, please specify which brand and leaf-type.

12. Please remember to record all snacks and drinks.

13. Once you have completed the 4-day diary, simply return it to us when you come back to the lab for your second testing session.

- If you do not know the exact quantity of food (in grams) or drink (in mL), please use the following tips to help you estimate these quantities:

1. **Household measures:** use cups, teaspoons, tablespoons.
2. **Visually:**

    - The Palm of your hand = 85g of meat or to describe the size of potatoes

    - Your fist = the same size as a cup. Use it to indicate amounts of Pasta, rice, cereal and fruit/veg

3. The tip of your thumb is a teaspoon – indicate sugar, marg, oils etc.

An example of a daily record of nutritional intake:

| DAY ONE | | | DATE 16.06.2011 | | OFFICIAL USE ONLY | |
|---|---|---|---|---|---|---|
| **Meal** | **Time** | **Food & Drink** | **Amount** | **Left-overs?** | **Food Code** | **Amount (g)** |
| **Early am** | 7am | Mug of tea, strong, milky, made with skimmed milk | 300ml (30ml milk) | | | |
| **Breakfast** | 7.30am | Bowl of Kellogg's branflakes,<br>Semi-skimmed milk.<br>Sliced banana.<br>White sugar.<br>Orange juice.<br>Mug tea, made as above. | Medium bowl.<br>½ pt milk.<br>Large<br>2 teaspoons<br>¼ pt.<br>300ml | | | |
| **Mid am** | 10am | Tesco finest white toast.<br>Flora margarine<br>2 glasses water.<br>Mug of filter coffee, black | 2 slices<br>Thinly applied<br>½ pt in total<br>300ml | | | |
| **Midday Meal** | 1pm | 2 crusty rolls.<br>Flora marg.<br>Mature Cheddar cheese<br>2 Jordan's cereal bar-fruit and nut.<br>1 can of Coca-cola.<br>1 glass water. | 2 medium-sized rolls.<br>Thinly applied<br>Thick chunks<br>2 x 60g bar.<br>330ml.<br>½ pt glass | | | |
| **Mid pm** | 3pm | 1 mug of filter coffee, as above.<br>3 custard cream biscuits (nutritional info attached). | 300ml.<br>3 biscuits. | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| **Evening Meal** | 6.30pm | New potatoes steamed in skins, Steamed broccoli,<br>Grilled fillet of salmon<br>with paprika.<br>2 low fat Ski strawberry yoghurt.<br>2 large glasses of white wine. | 7 small potatoes.<br>Handful.<br>130g (uncooked).<br>Pinch.<br>2 x 120g<br>2 x 250ml | 3 potatoes,<br>Salmon skin | | |
| **Evening Snack** | 9pm to 10.30pm | 2 mugs of tea<br>1 chocolate brownie (nutritional info attached)<br>1 large glass of Tesco's blackcurrant squash (no added sugar). | 2 x 300ml.<br>large<br>1 pt | | | |
| **Extras** | | Mars Bar | 1 standard size | | | |

Your daily record of nutritional intake:

| DAY ONE *Thursday* | | DATE | | | OFFICIAL USE ONLY | |
|---|---|---|---|---|---|---|
| **Meal** | **Time** | **Food & Drink** | **Amount** | **Left-overs ?** | **Food Code** | **Amount (g)** |
| **Early am** | | | | | | |
| **Breakfast** | | | | | | |
| **Mid am** | | | | | | |
| **Midday Meal** | | | | | | |
| **Mid pm** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Evening Meal** | | | | | | |
| **Evening Snack** | | | | | | |
| **Extras** | | | | | | |

NOTES:

Please record anything else which you may feel is relevant, e.g. illness

| **DAY TWO *Friday*** | | **DATE** | | | **OFFICIAL USE ONLY** | |
|---|---|---|---|---|---|---|
| **Meal** | **Time** | **Food & Drink** | **Amount** | **Left-overs ?** | **Food Code** | **Amount (g)** |
| **Early am** | | | | | | |
| **Breakfast** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Mid am** | | | | | | |
| **Midday Meal** | | | | | | |
| **Mid pm** | | | | | | |
| **Evening Meal** | | | | | | |
| **Evening Snack** | | | | | | |
| **Extras** | | | | | | |

NOTES:

Please record anything else which you may feel is relevant, e.g. illness

| **DAY THREE *Sunday*** | | **DATE** | | | **OFFICIAL USE ONLY** | |
|---|---|---|---|---|---|---|
| **Meal** | **Time** | **Food & Drink** | **Amount** | **Left-overs ?** | **Food Code** | **Amount (g)** |
| **Early am** | | | | | | |
| **Breakfast** | | | | | | |
| **Mid am** | | | | | | |
| **Midday Meal** | | | | | | |
| **Mid pm** | | | | | | |
| **Evening Meal** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| **Evening Snack** | | | | | | |
| **Extras** | | | | | | |

# Appendix IV – Physical Activity Readiness Questionnaire

**Readiness to exercise screening questionnaire**


| Participant ID: | | | Date: |
|---|---|---|---|
| | Age | Phone: | Email: |

Regular exercise is associated with many health benefits. Increasing physical activity is safe for most people. However, some individuals should check with a physician before they become more physically active. Completion of this questionnaire is a first step when planning to increase the amount of physical activity in your life. Please read each question carefully and answer every question honestly:

| | | |
|---|---|---|
| Yes | No | 1) Has a physician ever diagnosed you with a heart condition **and** indicated you should restrict your physical activity? |
| Yes | No | 2) When you perform physical activity, do you feel pain in your chest? |
| Yes | No | 3) When you were not engaging in physical activity, have you experienced chest pain in the past month? |
| Yes | No | 4) Do you ever faint or get dizzy and lose your balance? |
| Yes | No | 5) Do you have an injury or orthopaedic condition (such as a back, hip, or knee problem) that may worsen due to a change in your physical activity? |
| Yes | No | 6) Do you have high blood pressure or a heart condition in which a physician is currently prescribing a medication? |
| Yes | No | 7) Are you pregnant? |
| Yes | No | 8) Do you have insulin dependent diabetes? |

| | | |
|---|---|---|
| Yes | No | 9) Are you 69 years of age or older **and** not used to being very active? |
| Yes | No | 10) Do you know of any other reason you should not exercise or increase your physical activity? |
| Yes | No | 11) Are you currently on a medically prescribed diet? Explain |
| Yes | No | 12) Do you currently follow on specific diet restrictions (e.g. gluten free, vegetarian)? Explain |
| Yes | No | 13) Do you take any dietary supplements? If yes, please state what and the frequency |

If you answered yes to any of the above questions, talk with your doctor **before** you become more physically active. Tell your doctor your plan to exercise and to which questions you answer yes.

If you honestly answered no to all questions you can be reasonably certain you can safely increase your level of physical activity **gradually**.

If your health changes so you then answer yes to any of the above questions, seek guidance from a physician. I have read, fully understood and completed this questionnaire. The answers I have given are accurate to the best of my knowledge.

| | |
|---|---|
| Participant signature | Date |

# Appendix V – Habitual Physical Activity Questionnaire

Thank you for your interest in this research study. Prior to participation, we would like you to answer a few questions concerning your physical activity level. Please answer the following questions as honestly as you can.

Participant ID: Date:

1. What is your main occupation? _____

| | | | | | |
|---|---|---|---|---|---|
| 2. | At work I sit | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 3. | At work I stand | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 4. | At work I walk | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 5. | At work I lift heavy loads | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 6. | After work I am tired | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 7. | At work I sweat | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |

8. In comparison with others my own age I think my work is:

Much heavier ☐ Heavier ☐ As heavy ☐ Lighter ☐ Much lighter ☐

9. Do you play sport or exercise? Yes ☐ No ☐

If **YES**, which sport do you play most frequently? _____

| | | | | | |
|---|---|---|---|---|---|
| How long have you done? | Please write down as exact as possible year(s) and month(s) | | | | |
| How many hours per week? | Less than 1 ☐ | 1 to 2 ☐ | 2 to 3 ☐ | 3 to 4 ☐ | More than 4 ☐ |
| Time per session (hours) | ½ ☐ | 1 ½ ☐ | 2 ½ ☐ | 3 ½ ☐ | 4 ½ ☐ |
| How many months per year? | Less than 1 ☐ | 1 to 3 ☐ | 4 to 6 ☐ | 7 to 9 ☐ | More than 9 ☐ |

| | | | | | |
|---|---|---|---|---|---|
| What proportion of the month? | A few hours ☐ | A few days ☐ | 2 weeks ☐ | 3 weeks ☐ | Most of the month ☐ |

If you do a **second** sport (or exercise class), which is it? _____

| | | | | | |
|---|---|---|---|---|---|
| How many hours per week? | Less than 1 ☐ | 1 to 2 ☐ | 2 to 3 ☐ | 3 to 4 ☐ | More than 4 ☐ |
| Time per session (hours) | ½ ☐ | 1 ½ ☐ | 2 ½ ☐ | 3 ½ ☐ | 4 ½ ☐ |
| How many months per year? | Less than 1 ☐ | 1 to 3 ☐ | 4 to 6 ☐ | 7 to 9 ☐ | More than 9 ☐ |
| What proportion of the month? | A few hours ☐ | A few days ☐ | 2 weeks ☐ | 3 weeks ☐ | Most of the month ☐ |

10. Compared with others of my own age I think my physical activity during leisure time is:

Much more ☐ More ☐ The same ☐ Less ☐ Much less ☐

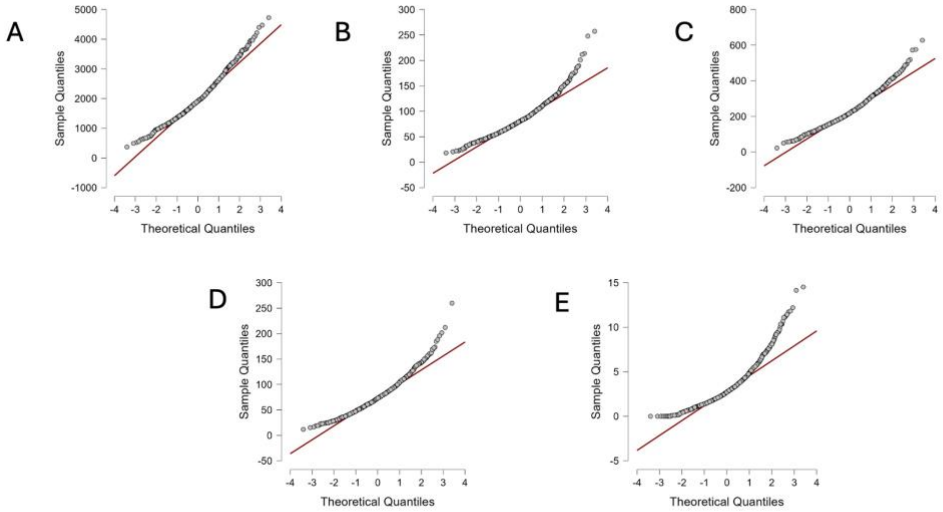
| | | | | | | |
|---|---|---|---|---|---|---|
| 11. | During leisure time I sweat | Very Often ☐ | Often ☐ | Sometimes ☐ | Seldom ☐ | Never ☐ |
| 12. | During leisure time I play sport | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 13. | During leisure time I watch TV | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 14. | During leisure time I walk | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |
| 15. | During leisure time I cycle | Never ☐ | Seldom ☐ | Sometimes ☐ | Often ☐ | Always ☐ |

16. How many minutes do you walk per day to and from work, school and/or shopping?

Less than 5 ☐ 5 to 15 ☐ 16 to 30 ☐ 31 to 45 ☐ More than 45 ☐

**Thank you for completing this questionnaire. All information will be kept strictly confidential.**

# Appendix VI – Supplementary data related to Chapter Three




Supplementary Figure 1. Q-Q plots illustrating the distribution of residuals for A. total energy intake, B. mean daily intake (MDI) protein, C. MDI carbohydrate, D. MDI fat, and E. MDI collagen

# Appendix VII – Supplementary form related to Chapter Six

## Female training study pre-participation form

**30 minutes**

Please read the participant information leaflet prior to completing the short form below, which will take about 5 minutes to complete. In Summary:

This form will ask questions about your current health status for screening purposes, including questions about your current reproductive hormone status. This is due to a known link between different levels of female reproductive hormones and collagen production, so we will need this information for our study on collagen supplementation. This information is confidential and will be assigned to your individual study ID number, and not your name, and cannot be requested by anyone.

* Required

1. Please enter your email address below. *
2. What is your date of birth? *
3. How tall are you? (cm) *
4. How much do you weigh? (kg) *
5. Are you currently injured, or recovering from an injury? *

- ◯ Yes
- ◯ No

6. If yes, please specify (injury location, injury type, time of injury) *

7. Which of the following best describes your menstruation status over the last 12 months?
*

- ◯ In the last 12 months, I have experienced a menstrual period every 21 to 35 days
- ◯ On at least one occasion in the last 12 months, the time between my menstrual periods has been less 23 days and/or greater than 35 days
- ◯ I have not experienced a menstrual period in the last 12 months

8. Are you currently using an oral contraceptive pill? *

- ◯ Yes
- ◯ No

9. Can you provide the name and dosage of the pill you are taking in the box below? *

10. Are you currently using any other form of hormone-based contraceptive? (e.g. injection, implant, patch, IUD coil) *

- ◯ Yes
- ◯ No

11. If yes, please specify the type of contraceptive, along with the brand and dosage *
12. Have you experienced any of the following symptoms in the last 6 months: Hot flashes, sleep problems, mood changes, bladder problems, changes in sexual function? (Yes or No, you will not be asked to specify) *

- ◯ Yes
- ◯ No

13. Are you currently using any form of HRT (Hormone Replacement Therapy)? *

- ◯ Yes
- ◯ No

14. If you answered yes to using HRT, can you please specify the form including type (i.e. combined HRT or oestrogen only), and mode of delivery (e.g. tablets, skin patch, implant, gel, spray). *

15. Have you read and understood the participant information leaflet? *

- ◯ Yes
- ◯ No
- ◯ I can't find the participant information leaflet

16. Do you consent to take part in the study? *

- ◯ Yes
- ◯ No