Effect of Collagen, Compared to Milk Protein, on Acute Recovery from Exercise Induced Muscle Damage Following Downhill Running

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Abstract

There is evidence to suggest that collagen can increase musculoskeletal connective tissue repair following exercise induced muscle damage (EIMD), however its effect(s) in contrast to a typical post-exercise supplement remains unclear. Therefore, this study compared the effect of collagen hydrolysate (CH) to milk protein concentrate (PRO) on acute recovery from EIMD, and subsequent delayed onset muscle soreness (DOMS), induced by downhill running. In a double-blind, randomised, independent group design study, 33 recreationally active males (18-40 years) consumed either CH or PRO, containing 25 g of protein, or an isoenergetic carbohydrate placebo (PLA) immediately post-exercise and once daily for three days. Indices of EIMD were measured before and 30 minutes, 24, 48, and 72 hours after 30 minutes of downhill running on a -15% slope at 80% of predetermined VO_2max speed. The protocol induced significant EIMD, with time effects (all P = < 0.001) for DOMS (visual analogue scale), countermovement jump, isometric midthigh pull, maximal voluntary isometric contraction, running economy, and biomarkers of muscle damage and inflammation. No group or interaction effects (P = < 0.05) were observed for any outcome measures, at any time point of recovery. The findings suggest the consumption of CH or PRO post-exercise does not improve indirect indices of EIMD during the acute recovery period following downhill running in recreationally active males.

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Abbreviations

A

AA	Amino acid
ACSM	American College of Sports Medicine
ALT	Alanine transaminase
AST	Aspartate transaminase

B

β-CTX	β-isomerized C-terminal telopeptide
β-NGF	Plasma beta-nerve growth factor
BPI-SF	Short-form brief pain inventory

C

CRP	C-reactive protein
CH	Collagen hydrolysate supplement
CON	Control group
CMJ	Countermovement jump
CK	Creatine kinase

D

DOMS	Delayed onset muscle soreness

E

EIMD	Exercise-induced muscle damage

H

hsCRP	High-sensitivity C-reactive protein
HRmax	Heart rate maximum

I

IL-1	Interleukin-1
IL-6	Interleukin-6
IL-10	Interleukin-10
IMTP	Isometric midthigh pull

L

LDH	Lactate dehydrogenase

M

MVDC	Maximal voluntary dynamic contraction
MVIC	Maximal voluntary isometric contraction
Mb	Myoglobin
MP	Milk protein

N

NPRS	Numeral pain rating scale

P

PLA	Placebo
PPO	Peak power output
PPT	Pressure pain threshold
PRO	Milk protein concentrate supplement

R

RSI	Reactive strength index

T

TNF-α	Tumour necrosis factor alpha

V

VAS	Visual analogue scale

VO_2max	Maximal oxygen consumption
VO_2peak	Peak oxygen consumption

W

WBC	White blood cell count
WP	Whey protein

#

1RM	One repetition maximum

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1. Introduction

Exercise-induced muscle damage (EIMD) is an acute ultrastructural muscular injury that is induced by modalities of exercise that are strenuous, repetitive, novel, and/or involve eccentric muscle contractions, such as downhill running (Owens et al., 2019). It is most notably characterised by symptoms that include delayed onset muscle soreness (DOMS), impaired muscular function, and an increase in biomarkers that indicate muscle damage and inflammation (Kanda et al., 2013). Among these, DOMS, which is a movement, stretch, and/or touch activated muscle soreness felt 24 to 48 hours after exercise has ceased, has received most research attention (Wang et al., 2022). Whilst EIMD is temporary and beneficial for future muscle adaptation (Bongiovanni et al., 2020), it can still create several problems for regular exercisers by impairing exercise performance during subsequent bouts of exercise (Hody et al., 2019) and/or by causing a disruption to normal daily activities (Tesarz et al., 2012). Therefore, a rapid recovery from EIMD, and subsequent DOMS, is very important.

Although significant progress has been made to find recovery modalities that alleviate the symptoms of EIMD, the answer to the question “what is the best nutritional intervention for expediting acute recovery from EIMD” is not clear (Cheung et al., 2012). One nutritional intervention at the forefront of most recommendations for the treatment of EIMD is dairy protein (Robberechts et al., 2023). The consumption of dairy protein following EIMD is believed to improve recovery through the delivery of essential amino acids that enhance rates of muscle protein synthesis, which promotes muscle repair (West et al., 2017). While some existing studies have previously found evidence to support a beneficial effect of dairy protein on markers of EIMD, including muscular function (Brown et al., 2018; Buckley et al., 2010; Cooke et al., 2010), others have not (Gee et al., 2019; Nieman et al., 2020). Moreover, the majority of past research has indicated that it does not reduce exercise-induced DOMS (Apweiler et al., 2019; Aussieker et al., 2023; Betts et al., 2009; Brown et al., 2018; Buckley et al., 2010; Cockburn et al., 2008; Dahlstrom Burnley et al., 2010; Davies et al., 2020; Eddens et al., 2017; Etheridge et al., 2008; Gee et al., 2019; Hilkens et al., 2021; Nieman et al., 2020; Ormsbee et al., 2022; Saracino et al., 2020; White et al., 2008). Alternatively, researchers are now examining the effect of other protein sources on recovery from EIMD, including collagen.

Sourced from the underutilised by-products of the meat industry, collagen is a lactose-free protein high in non-essential amino acids that are used in the synthesis of collagen (Prowting et al., 2021). Traditionally applied in the context of anti-aging (Brandao-Rangel et al., 2022), it is believed to benefit exercise recovery by enhancing rates of collagen synthesis in the damaged connective tissue (Shaw et al., 2016). Indeed, collagen has been shown to attenuate several markers of EIMD (Lopez et al., 2015), most notably DOMS (Clifford et al., 2019). Nonetheless, few studies have examined its effects on recovery from EIMD, and an even smaller number have directly compared it to dairy protein (Aussieker et al., 2023; Robberechts et al., 2023).

Therefore, based on the conflicting evidence but continued popularity of dairy protein, and the developing literature on collagen, the aim of this study was to compare the effect of collagen hydrolysate (CH) to milk protein concentrate (PRO) on acute recovery from EIMD, and subsequent DOMS, induced by downhill running in recreationally active males. It was hypothesised that 25 g of protein from CH would reduce ratings of DOMS, increase recovery for measures of muscular function, and attenuate biomarkers of muscle damage and inflammation following EIMD, to an equal or greater extent than an equivalent amount of protein from PRO, and that all protein sources would be more effective than a placebo (PLA).

Our main purpose was to not only contribute to the gap in the existing literature, but through our contribution we hope to equip experts in the field, including exercise practitioners that make important recommendations to regular exercisers, with tangible solutions that reduce/minimise the problems caused by EIMD. The overall impact of our findings, in turn, may also provide additional evidence for the utilisation of collagen, a by-product created by the meat industry.

The thesis begins by situating the current study in the related literature on EIMD, before covering the effects of dairy protein and collagen (Chap. 2). Following the literature review, the aims and hypotheses are introduced (Chap. 3), along with the methods used to compare the effects of CH to PRO on acute recovery from EIMD, and subsequent DOMS, following downhill running (Chap. 4). The results are then displayed (Chap. 5) before a discussion of the findings, in relation to similar research, and an acknowledgement of strengths, limitations, and recommendations for future research are presented (Chap. 6). The thesis will end with a conclusion that outlines some important implications and how our findings add to the existing literature on EIMD (Chap. 7).

2. Literature Review

2.1 Literature Overview

Modalities of exercise that are unaccustomed, strenuous, repetitive, and/or involve muscle lengthening frequently cause EIMD (Tanabe et al., 2022). This can lead to several symptoms including DOMS, impaired muscular function, and the presence of muscle damage and inflammatory biomarkers (Kanda et al., 2013); although the presentation and severity of these symptoms are often determined by individual characteristics, such as training history (Howatson et al., 2008). In most cases, EIMD resolves naturally in time without any intervention or specialist treatment (Cheung et al., 2003). Adaptation also occurs, where the muscles appear to be more resilient to future damage upon their repeated exposure to exercise (Bongiovanni et al., 2020). The symptoms or markers of EIMD also serve a practical purpose, becoming an exercise deterrent that permits an adequate time to recover (Hody et al., 2019). However, EIMD can still create multiple problems for regular exercisers when it impairs subsequent exercise performance (Hody et al., 2019) and/or causes a disruption to normal daily activities (Tesarz et al., 2012).

Researchers have examined the possible causes of EIMD, and while these are not entirely clear (Cheung et al., 2003), the two-phase model of ultrastructural muscle damage and inflammation has been generally accepted to be the principal cause (Peake et al., 2016). Studies have also emphasised the role of the extracellular matrix (connective tissue network surrounding muscles) and how its damage often contributes to the symptoms of EIMD, most notably DOMS (Mizumura & Taguchi, 2016; Peake et al., 2016; Wilke & Behringer, 2021). In order for researchers to understand the phenomenon that is EIMD, including its symptoms and mechanisms, it is induced and consequently assessed in a controlled environment using a variety of standardised exercise protocols and measures. Purposefully inducing and measuring EIMD also allows researchers to examine the effect of a variety of nutritional interventions that may or may not expedite its recovery (Bongiovanni et al., 2020).

Dairy protein is one of the most widely recommended post-exercise supplements on the market (Master & Macedo, 2021). Largely exerting its effects on recovery from EIMD through muscle protein synthesis, its consumption is thought to provide the body with essential amino

acids that cannot otherwise be endogenously sourced (Aussieker et al., 2023). The effect of dairy protein on muscular function following EIMD has been noteworthy, with many studies reporting an improvement in exercise performance recovery (Brown et al., 2018; Buckley et al., 2010; West et al., 2017). Conversely, however, past research has also failed to observe a benefit (Betts et al., 2009; Gee et al., 2019; Nieman et al., 2020; ten Haaf et al., 2021). Alongside inconsistent findings for muscular function, a number of existing studies have also found that dairy protein supplementation has no effect on DOMS following EIMD (Apweiler et al., 2019; Betts et al., 2009; Brown et al., 2018; Buckley et al., 2010; Cockburn et al., 2008; Eddens et al., 2017; Etheridge et al., 2008; Gee et al., 2019; Hilkens et al., 2021; Nieman et al., 2020; Ormsbee et al., 2022).

Although the effect of dairy protein in the context of exercise has been thoroughly examined, less is known about the effect of collagen supplementation (Robberechts et al., 2023). Collagen is a lactose-free protein alternative that is an inexpensive waste by-product of the meat industry (Pap et al., 2022). The consumption of collagen may assist in recovery from EIMD through the provision of non-essential amino acids used in the synthesis of new collagen within the extracellular matrix (Shaw et al., 2016). Contrary to the findings from dairy protein, previous studies have found both an improvement in muscular function, as well as a reduction in DOMS following collagen supplementation (Clifford et al., 2019; Lopez et al., 2015).

Given the conflicting evidence of dairy protein, but continued popularity, and the developing literature on collagen supplementation, it is surprising that only a limited number of studies have examined collagen's effect on EIMD, and an even fewer number have directly compared it to dairy protein. Therefore, the aim of this literature review was to compare the effects of collagen to dairy protein on acute recovery from EIMD.

2.2 EIMD

An exercisers' participation in unaccustomed, strenuous, repetitive, and/or eccentric exercise often causes EIMD (Kanda et al., 2013). A variety of indirect markers are used to define EIMD, which may include, but are not limited to, DOMS, impaired muscular function, and an increase in muscle damage and inflammatory biomarkers (Figure 2.1; Markus et al., 2021). What causes EIMD is largely disputed, with many mechanisms having been considered to be responsible (Allen et al., 2004), although based on growing evidence, the two-phase model of ultrastructural muscle damage, followed by inflammation, has been well-received by experts in the field as a

likely cause (Peake et al., 2016). The contribution of damage to the extracellular matrix in EIMD has also been recently emphasised by researchers (Clifford et al., 2019; Prowting et al., 2021). However, in order to gather evidence in support for these proposed mechanisms, as well as answers to important questions, such as "what is the best nutritional intervention for expediting acute recovery from EIMD", first EIMD has to be successfully induced and adequately assessed in a controlled laboratory environment using a standardised EIMD protocol, and a combination of valid and reliable measures.

2.2.1 Markers of EIMD

2.2.1.1 DOMS

DOMS is a marker of EIMD that receives a great deal of attention due to many researchers not precisely understanding why or how it occurs (Cheshier & Jacobson, 2021; Mizumura & Taguchi, 2024). Further adding to its ambiguity, while it may be somewhat useful for defining EIMD, it does not always occur and the timeline of presentation to recovery is often highly variable between different people (Nosaka et al., 2006). DOMS is a type of exercise-induced hyperalgesia that can be described as a dull, achy, or tender feeling that is localised to the affected muscles (Mizumura & Taguchi, 2024). It emerges as a delayed pain response, sometimes paired with swelling or stiffness, which slowly increases in intensity until peaking 24 to 48 hours after exercise (Wilke & Behringer, 2021). Most notably occurring after eccentric exercise, it is distinct from other sensations of pain because it is touch, stretch, and/or contraction activated and is typically not active during rest or while the muscles are relaxed (Kanda et al., 2013). On a continuum, the pain can be mild to more intense, but fortunately it dissipates within a few days unless someone is a novice exerciser (Mizumura & Taguchi, 2024). In this case, novices often feel a more intense and lasting pain until their exposure to exercise increases, which lessens the subsequent intensity and duration of DOMS (Contrò et al., 2016). In conjunction to training history, there are also other predetermining factors that may increase or decrease someone's susceptibility to DOMS (e.g., age, sex & duration of exercise; Bongiovanni et al., 2020).

Despite being temporary, DOMS can be problematic for regular exercisers for multiple reasons. Firstly, it may impair subsequent exercise performance due to the psychological aspect of wanting to avoid further pain/discomfort that is activated when trying to use the affected muscles

(Hody et al., 2019). Furthermore, any swelling and/or stiffness that accompanies the pain may also reduce exercise performance. Additionally, outside of exercise, DOMS can cause a disruption to normal daily activities (e.g., sitting on the toilet) that are otherwise easy to perform when pain is not a limiting factor (Cheshier & Jacobson, 2021).

2.2.1.2 Impaired Muscular Function

Another marker of EIMD that also negatively affects exercise performance is impaired muscular function (Kristoffersen et al., 2022). It is one of the more observable markers that, according to some, is the best way to measure EIMD (Owens et al., 2019). Impaired muscular function may include a loss of force or power output, limited range of motion, and/or altered neuromuscular control (e.g., reduced firing frequency or motor unit recruitment; Byrne et al., 2000). It occurs much earlier than DOMS, where the peak magnitude is highest immediately following or soon after exercise when neurological fatigue and reduced proprioception are also present (Allen et al., 2004). As the initial fatigue subsides, the impairment primarily becomes a consequence of ultrastructural muscle damage and metabolic changes that increase passive tension and impede muscle contraction (Byrne et al., 2000). Connective tissue damage may also contribute to the loss of muscular function after EIMD by reducing the transmission of force (Wilke & Behringer, 2021). All of this is then further perpetuated by the inflammatory response (Kristoffersen et al., 2022).

Fortunately, similar to DOMS, impaired muscular function following EIMD is also temporary. However, if the impairment is greater than a 20% loss, recovery back to baseline typically takes longer even after DOMS has ceased (Fatouros & Jamurtas, 2016). Furthermore, besides being a physiological barrier by interfering with exercise performance (e.g., reducing maximal force, power output, running economy etc.) during subsequent bouts of exercise, impaired muscular function also acts as a psychological barrier for the progression of a training program (Kristoffersen et al., 2022).

2.2.1.3 Muscle Damage and Inflammatory Biomarkers

Alongside DOMS and impaired muscular function, an increase in muscle damage and inflammatory biomarkers are also used to define EIMD (Allen et al., 2004). EIMD causes

intramuscular proteins, such as creatine kinase (CK), myoglobin (Mb), and lactate dehydrogenase (LDH), normally contained inside the muscle cell, to leak out and into circulation through an increase in membrane permeability, caused by structural and metabolic disturbances that trigger several inflammatory responses (Tanabe et al., 2022). The presence, peak magnitude, and consequential clearance of these larger proteins or 'biomarkers of muscle damage' are dependent on a multitude of factors, which may include individual characteristics such as age, sex, and genetics, or the chosen modality of exercise, including its duration and selected intensity (Braird et al., 2012). Furthermore, whist increases in circulatory biomarkers of muscle damage might be used by researchers to assess EIMD, they are not as reliable as muscular function measures (Pompermayer et al., 2021).

Occurring at the same time or soon after the leakage of intramuscular proteins, the inflammatory cytokines interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF- α), and enzyme C-reactive protein (CRP) are also released into circulation (Chrismas et al., 2018). Produced by damaged muscle fibres and/or immune cells (e.g., leucocytes, neutrophils, and macrophages), they are important mediators of the inflammatory response that appropriately amplify or supress this response when or where necessary (Kanda et al., 2013). Similar to biomarkers of muscle damage, the release of inflammatory biomarkers is also subject to individual characteristics and exercise variables (Arroyo et al., 2017), and their reliability as a marker of EIMD may not be as strong as others; particularly when the EIMD protocol fails to trigger a significant inflammatory response (Buckley et al., 2010; Robberechts et al., 2023).

An increase in biomarkers of muscle damage and inflammation in circulation does not directly cause problems to the exerciser in the same way as DOMS or impaired muscular function. This does, however, provide an indication of whether any ultrastructural muscle damage and/or inflammation are present, which are the primary causes responsible for the reduction in exercise performance during subsequent exercise bouts and the disruption to normal daily activities (Stožer et al., 2020).

2.2.2 Mechanisms of EIMD

While many mechanisms have been suggested to be the principal cause of EIMD (Markus et al., 2021), there has been a growing acceptance among experts in the field for the two-phase model of ultrastructural muscle damage and inflammation (Figure 2.1; Stožer et al., 2020), with an

emphasis on damage to the extracellular matrix (Bongiovanni et al., 2020; Wilke & Behringer, 2021). Though it might be easier or more straightforward to categorise EIMD into two distinct phases, the phenomenon that is EIMD is highly complex, as these phases may not occur simultaneously, nor separately, if they happen to occur at all, and their contribution to the problems caused by EIMD (impaired muscular function and DOMS) is also complicated (Allen et al., 2004).

Figure 2.1

Two-Phase Model of EIMD

Note. Figure sourced from Stožer et al. (2020).

2.2.2.1 Ultrastructural Muscle Damage

Ultrastructural muscle damage is the primary phase of the two-phase model of EIMD (Stožer et al., 2020). Mechanical stress from exercise induces damage that disrupts normal biological functioning at many organisational levels, from inside the muscle cell to outward effects on the musculoskeletal system as a whole (Stožer et al., 2020). One of the most important myocellular components affected are sarcomeres, the smallest functional unit of the cell (Allen et al., 2004). Upon their initial exposure to force, stronger sarcomeres are able to resist the stretch and can re-interdigitate easily after the muscle relaxes (Proske & Allen, 2005). However, those weakest in a series, particularly on fast twitch fibres with weaker Z-lines and more rigid filaments, progressively wane, taking on more and more of the stretch until they become disrupted (Wilke & Behringer, 2021). As the stretch continues, stronger sarcomeres can no longer resist the stretch and disruption proceeds down the length of the myofibril (Stožer et al., 2020). Sarcomere disruption particularly occurs during muscle lengthening, where the eccentric force exerted on the fewer number of muscle fibres recruited is greater than that exerted on the numerous amount of muscle fibres recruited during muscle shortening (Tenberg et al., 2022).

Other myocellular components are also affected following their exposure to mechanical stress during exercise. This includes the sarcolemma, where various membrane proteins (e.g., desmin and dystrophin) that are important for its functionality become dislodged after EIMD (McKune et al., 2012). Mechanical stress has also been shown to affect mitochondria, reducing their number and changing their normal distribution (Ying et al., 2021); although this might be due to the following build-up of calcium or oxidative damage, rather than their initial exposure to mechanical stress (Li et al., 2021).

Consequently, the structural alterations mentioned above prompt several metabolic changes within the cell to occur (Proske & Allen, 2005). Firstly, sarcomere overstretch interferes with the normal functioning of the sarcoplasmic reticulum and opens stretch-activated ion channels, which causes the accumulation of intracellular calcium ions in the sarcoplasm and excitation-coupling dysfunction (Stožer et al., 2020). This may then trigger different pathways (e.g., calpain proteolytic pathway) that further proliferate muscle damage (McKune et al., 2012). Furthermore, the build-up of intracellular calcium can activate proteolytic enzymes (e.g., metalloproteinases) to degrade healthy cellular components (Cheung et al., 2003), and impair mitochondrial functioning (e.g., induce their swelling and cause apoptosis; Li et al., 2021).

The contribution of the first phase in the two-phase model of EIMD towards the problems caused by EIMD is significant, primarily regarding the loss of muscular function. Alongside neural fatigue, according to McKune et al. (2012), ultrastructural muscle damage is one of the main causes of impaired muscular function following EIMD. Although it might be evident how structural and metabolic changes within the muscle cell impair force production (Tanabe et al., 2022), the contribution towards sensations of DOMS is not as clear (Cheung et al., 2003). While some research suggests ultrastructural muscle damage (lesions and Z-line streaming) may invoke muscle pain by stimulating mechanoreceptors (Cheung et al., 2003), the magnitude of damage does not always simultaneously match that of DOMS (Wilke & Behringer, 2021). Nonetheless, the first phase may still provide part of the explanation for why DOMS occurs (Cheung et al., 2003).

2.2.2.2 Inflammation

The secondary phase in the two-phase model of EIMD is inflammation (Stožer et al., 2020). Activated by ultrastructural muscle damage, the inflammatory response plays an important role in muscle repair and regeneration under normal conditions, but it may further proliferate damage and lengthen the time taken to recover (Owens et al., 2019). Inflammation involves a series of tightly linked sequential events (McKune et al., 2012). Firstly, pro-inflammatory cytokines are released in response to the damage, which signal specialised immune cells (e.g., neutrophils and macrophages) towards the site of injury. After infiltrating the area, these cells phagocytose necrotic cells and clear any cellular debris (Lin et al., 2022), whilst simultaneously stimulating the activity of satellite cells involved in muscle repair (Stožer et al., 2020). However, harmful by-products of their important work are produced, including reactive oxygen and nitrogen species that worsen myocellular damage, if their production exceeds their clearance (Kim et al., 2022). Furthermore, immune cell overactivity may inhibit repair and contribute to myocellular death (Charzaud et al., 2009). Evidence of the inflammatory phase has been demonstrated by a 31% increase in circulating leucocytes and a 44% increase in neutrophils following EIMD induced by calf raises (Kanda et al., 2013). Furthermore, an efflux of inflammatory biomarkers in circulation also supports this phase (Stožer et al., 2020).

The inflammatory response has been shown to contribute to several of the problems caused by EIMD (Caballero-Garcia et al., 2023). Firstly, it perpetuates the loss of muscular function by proliferating ultrastructural muscle damage (Stožer et al., 2020). Inflammation often leads to

swelling and an increase in intramuscular pressure, which may compress blood vessels carrying oxygen and nutrients, further impeding muscle contraction (McKune et al., 2012). Swelling can also exert pressure on nociceptors and contribute to DOMS (Wilke & Behringer, 2021); although the timeline of both may not always correspond (Yu et al., 2013). Lastly, the inflammatory response also promotes the release of histamine, prostaglandins, and substance P, which may aggravate nerve endings in the extracellular matrix that are particularly sensitive to chemical and noxious stimuli, contributing to DOMS (Wilke & Behringer, 2021; Mizumura & Taguchi, 2016).

2.2.2.3 Extracellular Matrix Damage

Mechanical stress from exercise may induce ultrastructural muscle damage that triggers an inflammatory response, but muscle cells are not affected in isolation (Wilke & Behringer, 2021). Due to their close structural connection, the surrounding extracellular matrix also sustains damage (Figure 2.2; Bongiovanni et al., 2020). Often overlooked in conventional 'two phase' models of EIMD (Connolly et al., 2003), the extracellular matrix is a dense fibrous sheath of connective tissue that permeates individual muscle fibres (endomysium), bundles of muscle fibres (perimysium), and covers the entirety of the muscle (epimysium; Wilke & Behringer, 2021). Acting as a supportive, scaffold-like network, containing a vast number of collagen fibrils, proteoglycans, and glycoproteins, it bridges the gap between individual muscle cells and supports their normal functioning (e.g., signalling, differentiation, migration, regulation & proliferation; Zhang et al., 2021). During exercise, mechanical stress causes microtears or lesions to occur in the extracellular matrix (Lopez et al., 2015), particularly at the musculotendinous junction (Wilke & Behringer, 2021). This weakens its structural integrity and shifts the normal alignment of muscle cells, impacting the transmission of force (Tenberg et al., 2022). Evidence of extracellular matrix damage following EIMD is supported by medical imaging showing edema and stiffness of the deep myofascial tissue (Fu et al., 2024), and by an increase in circulatory markers of collagen degradation, such as β-isomerized C-terminal telopeptide (β-CTX; Clifford et al., 2019).

Figure 2.2

Two-Phase Model of EIMD with an Emphasis on Extracellular Matrix Damage

Note. Figure sourced from Bongiovanni et al. (2020).

The contribution of extracellular matrix damage to the problems caused by EIMD is also significant. Once damaged, the extracellular matrix impairs muscular function through a reduction in force transmission (Tenberg et al., 2022). It also becomes stiffer and less pliable, restricting normal joint range of motion and also impairing muscular function. Extracellular matrix damage is also one of the main factors responsible for DOMS, as it is an area densely innervated with sensory afferents and free nerve endings (Mizumura & Taguchi, 2016). However, not all evidence of its damage correlates with the timeline of DOMS (Wilke & Behringer, 2021). Research is also yet to find evidence for a cause and effect relationship (Tenberg et al., 2022).

2.2.3 EIMD Protocols

One approach researchers use to increase their understanding of the markers and mechanisms of EIMD is to induce damage in a controlled laboratory environment. There is a range

of validated, standardised, and replicable protocols for them to choose from, however consensus on which are effective and which are not is minimal at the very least, as displayed by the considerable number of different protocols that have been used (Table 2.1). Nonetheless, among those that have been shown to be effective at inducing EIMD are drop jumps (Clifford et al., 2019), maximal eccentric knee contractions (White et al., 2008), and downhill running (Etheridge et al., 2008).

Table 2.1

EIMD Protocols used in Collagen and Dairy Protein Studies

Author(s)	EIMD Protocol	Was EIMD Induced?
Aussieker et al. (2023)	Barbell squats (15, 12, 10, 10, 8 & 8 at 60% 1RM)	Yes
Apweiler et al. (2019)	Drop jumps (4 x 25 reps)	Yes
Betts et al. (2009)	90-min intermittent shuttle run at VO_2max	Yes
Bischof et al. (2024)	Drop jumps (6 x 25 reps)	Yes
Brown et al. (2018)	30m sprints with rapid 10m deceleration phase (15 reps)	Yes
Buckley et al. (2010)	Maximal eccentric knee extensions (100 reps)	Yes
Clifford et al. (2019)	Drop jumps (6 x 25 reps)	Yes
Cockburn et al. (2008)	Isokinetic unilateral knee extensions (6 x 10 reps)	Yes
Cockburn et al. (2010)	Isokinetic unilateral knee extensions (6 x 10 reps)	Yes
Cooke et al. (2010)	Leg press, leg extension & leg flexion (4 x 10 reps of each)	Yes
Dahlstrom Burnley et al. (2010)	Maximal eccentric knee extensions (10 x 10 reps)	Yes
Davies et al. (2020)	Barbell squats (10 x rep to failure at 70% 1RM)	Yes
Draganidis et al. (2017)	Eccentric knee extensions (20 x 15 reps)	Yes
Eddens et al. (2017)	High intensity stimulated cycling & drop jumps (5 x 20 reps)	Yes
Etheridge et al. (2008)	30-min downhill run (-10% at 75% age predicted HRmax)	Yes
Gee et al. (2019)	Barbell squats, bench press, deadlift & military press (4 x 8 reps at 75% 1RM)	Yes
Hilkens et al. (2021)	Drop jumps (10 x 10)	Yes
Hirose et al. (2013)	Calf raises (5 x 15 reps of body weight)	Yes
Lopez et al. (2015)	Bench press (8 x rep to failure at 75% of body weight)	Yes
Nieman et al. (2020)	90-min eccentric-exercise bout (incl. a downhill run)	Yes
Ormsbee et al. (2022)	Barbell squat & bench press (8 x 8 reps, set 1: 55% 1RM, set 2: 65% & set 3-8: 75% 1RM)	No
Prowting et al. (2021)	Drop jumps (5 x 20 reps)	Yes
Robberechts et al. (2023)	3-week eccentric-exercise training (incl. knee extensions, one-leg squats & drop jumps)	Yes
Saracino et al. (2020)	Maximal eccentric knee extensions & flexions (5 x 15 reps of each)	Yes
ten Haaf et al. (2021)	15-km run	No
West et al. (2017)	Bench press + pulldown, overhead press + seated row, leg press + leg extension (4 x 10 reps at 75% 1RM)	Yes
White et al. (2008)	Maximal eccentric knee extensions (5 x 10 reps)	Yes

Note. One rep (repetition) maximum (1RM), heart rate maximum (HRmax), maximal voluntary isometric contraction (MVIC). EIMD based on observed time effect(s) for markers of EIMD.

2.2.3.1 Drop Jumps

Drop jumps are an eccentric-concentric movement, where a participant 'drops' off a box or platform from a set height into a maximal vertical jump (Clifford et al., 2019). Upon their landing, a high amount of mechanical force is transmitted throughout the muscles of the lower body, which forcibly lengthens them under extreme tension (de Lima et al., 2018). Drop jumps require minimal equipment, expertise, and space, and have been shown to effectively induce muscle damage in a laboratory (Bischof et al., 2024; Clifford et al., 2019). For example, 100 drop jumps caused a significant change over time for DOMS and all measures of muscular function in recreationally active males (Hilkens et al., 2021). Similarly, a drop jump protocol used by Prowting et al. (2021) also induced EIMD in resistance-trained males. While drop jumps, according to some researchers, are a sport-specific exercise that reflects movements that cause damage in real-world contexts (Prowting et al., 2021), the volume traditionally used in a laboratory typically exceeds that which is normally involved in training or competition settings; particularly when more drop jumps are required to induce muscle damage in well-trained participants (de Lima et al., 2018). Drop jumps also require explosive power and place a great deal of stress on the joints, which may only make them suitable to induce EIMD in those without pre-existing joint issues.

2.2.3.2 Maximal Eccentric Knee Contractions

Maximal eccentric knee contractions are also used by researchers to induce EIMD in a controlled laboratory environment (Table 2.1). While seated in an isokinetic dynamometer or similar machine, participants perform a set number of maximal eccentric knee extensions under a load to induce muscle damage in the quadriceps (Buckley et al., 2010). Many researchers have previously demonstrated this protocols' effectiveness at inducing EIMD (Dahlstrom Burnley et al., 2010). For example, 100 maximal eccentric knee contractions, used by Buckley et al. (2010) to induce EIMD in sedentary males, successfully caused a decrease in force and an increase in DOMS. Similarly, White et al. (2022) also found them useful to induce EIMD in sedentary males. Maximal eccentric knee contraction protocols are generally highly standardised and replicable, particularly

when isokinetic dynamometers apply a constant load, at a constant speed, over a specific range of motion (de Araujo Ribeiro Alvares et al., 2015). However, this method of muscle damage induction may lack real-world applicability, as it is not normally induced this way naturally.

2.2.3.3 Downhill Running

Downhill running is another protocol that is used to induce EIMD. Researchers mirror outdoor downhill running by using a motorised treadmill that is set on a decline with the belt put into reverse (Etheridge et al., 2008). Downhill running involves high force, repetitive muscle lengthening, under extreme tension. It is a strenuous form of exercise, where the muscles have to act as brakes at an extended range of motion to stop the body's acceleration forward (Bontemps et al., 2020). It has been shown to successfully cause significant EIMD in multiple studies (Braun & Dutto, 2003; Chen et al., 2007; Chen et al., 2009; Lima et al., 2019; Lima et al., 2021; Philpott et al., 2023; Southall-Edwards et al., 2022). For example, Etheridge et al. (2008) found a 30-minute downhill run at 75% of age predicted heartrate max on a -10° slope increased muscle soreness and decreased muscular function in recreationally active males. While downhill running might be an effective means to induce EIMD, it is not a widely used exercise protocol (Table 2.1). Its lack of use in favour of other protocols might be explained by the physically demanding nature of downhill running (Southall-Edwards et al., 2022). However, it can be useful in instances where it is harder to induce EIMD, specifically in regards to well-trained individuals that can tolerate the high amount stress placed on their joints during this type of protocol (Bontemps et al., 2020). Lastly, downhill running does not depend on a participants' level of motivation or fatigue in the same way that other EIMD protocols do; many require continuous effort throughout the protocol to ensure damage occurs.

2.2.4 Measures of EIMD

Once EIMD has been effectively induced, researchers use a number of measures to assess and compare before and after exercise changes in markers of EIMD (Table 2.2). This may include standardised measures of muscle soreness, exercise performance, and blood sampling (Markus et al., 2021), which enables them to perform a comprehensive assessment of EIMD. Although corresponding to the wide range of EIMD protocols used, researchers often disagree on what

measures are most appropriate, as apparent by few studies using the same combination of measures (Table 2.2).

Table 2.2

Measures of EIMD used in Collagen and Dairy Protein Studies

			Biomarker(s)	
Author(s)	Muscle Soreness	Muscular Function	Muscle Damage	Inflammation
Aussieker et al. (2023)	Likert scale	-	-	-
Apweiler et al. (2019)	VAS & PPT	MVIC & CMJ	-	-
Betts et al. (2009)	VAS	MVIC	CK, Mb & LDH	IL-6, -10, -1 & CRP
Bischof et al. (2024)	-	-	Mb & LDH	hsCRP
Brown et al. (2018)	VAS & PPT	MVIC, CMJ, sit/reach, RSI & 30m sprint	CK	-
Buckley et al. (2010)	VAS	MVIC	CK	TNF-α
Clifford et al. (2019)	VAS & PPT	MVIC & CMJ	CK, LDH AST & ALT	IL-6, β-NGF & WBC
Cockburn et al. (2008)	VAS	Isokinetic torque	CK & Mb	-
Cockburn et al. (2010)	VAS	Isokinetic torque & RSI	CK	-
Cooke et al. (2010)	-	MVIC & isokinetic torque	CK & LDH	-
Dahlstrom Burnley et al. (2010)	Pain scale	Isokinetic torque, max reps & average power	CK	-
Davies et al. (2020)	VAS	CMJ & isometric squat force	CK	-
Draganidis et al. (2017)	VAS	Isokinetic torque	CK	WBC, NF-κB,
Eddens et al. (2017)	VAS	MVIC, CMJ & 16.1km cycle time trial	CK	CRP
Etheridge et. (2008)	VAS & PPT	MVIC & PPO (cycle ergometer)	CK	-
Gee et al. (2019)	VAS	Isokinetic torque, CMJ & seated medicine ball throw	-	-
Hilkens et al. (2021)	VAS	MVIC, CMJ & isokinetic torque	CK	CRP
Hirose et al. (2013)	VAS	-	CK	-
Lopez et. (2015)	VAS	Upper-body-resistance-challenge	CK & LDH	CRP
Nieman et al. (2020)	VAS	Bench press until failure, vertical jump test, mid-thigh pull & 30s Wingate	CK, LDH, Mb, AST & ALT	CRP
Ormsbee et al. (2022)	VAS	1RM (bench press & squat)	CK	CRP
Prowting et al. (2021)	VAS	MVIC & CMJ	-	-
Robberechts et al. (2023)	VAS	MVIC, MVDC & CMJ	CK	IL-6
Saracino et al. (2020)	VAS	MVIC & isokinetic torque	CK	IL-6
ten Haaf et al. (2021)	NPRS, PPT & BPI-SF	-	CK & LDH	-
West et al. (2017)	-	MVIC, CMJ, reps to failure & 30s Wingate	-	-

Author(s)	Muscle Soreness	Muscular Function	Biomarker(s)	
			Muscle Damage	Inflammation
White et al. (2008)	VAS	MVIC	CK	-

Note. Visual analogue scale (VAS), pressure pain threshold (PPT), maximal voluntary isometric contraction (MVIC), counter-movement jump (CMJ), creatine kinase (CK), myoglobin (Mb), lactate dehydrogenase (LDH), interleukin-6, (IL-6), interleukin-10 (IL-10), interleukin-1 (IL-1), C-reactive protein (CRP), high-sensitivity C-reactive protein (hsCRP), reactive strength index (RSI), tumour necrosis factor alpha (TNF-α), aspartate transaminase (AST), alanine transaminase (ALT), plasma beta-nerve growth factor (β-NGF), white blood cell count (WBC), peak power output (PPO), one repetition (rep) maximum (1RM), maximal voluntary dynamic contraction (MVDC), numeric pain rating scale (NPRS), short-form brief pain inventory (BPI-SF).

2.2.4.1 Muscle Soreness

A variety of muscle soreness measures have been created to assess exercise-induced DOMS. Among those most commonly used by researchers is a visual analogue scale (VAS; Table 2.2). VAS is a self-assessment that requires participants to rate their muscle soreness from 0 (no muscle soreness) to 10 (extreme muscle soreness) on a line of a fixed length (e.g., 100 to 200 mm; Lau et al., 2013). The distance of their rating from 0 is measured in mm to produce a numeral score for quantitative analysis (Clifford et al., 2019). Ratings of muscle soreness using a VAS vary based on exercise variables (e.g., intensity or duration), in addition to individual characteristics (Contrò et al., 2016). VAS is an easily implemented measure of muscle soreness that is a feasible way to track changes in DOMS over time (Cleather & Guthrie, 2007). However, it is a one-dimensional rating that only captures intensity and disregards other important nuances, including the emotions muscle soreness incites (Cleather et al., 2007). It is also a fixed rating from 0 to 10, where a participant could feel more muscle soreness than they are able to rate but are restricted by a definitive number (Nosaka et al., 2006). Furthermore, some experts in the field have posed questions around whether it is even possible to quantify DOMS using measures of muscle soreness, as it is inherently subjective and personal (Nosaka et al., 2006). There is also a lack of standardisation around the implementation of VAS because, quite often, different muscle actions (e.g., squat or leg extension) are used (if any) to activate muscle soreness (Lau et al., 2013).

Alternatively, muscle soreness can be assessed with a pressure pain threshold (PPT) test (Chesterton et al., 2003). Using a handheld algometer, increasing levels of pressure (N) are applied to an area of the muscle (generally the muscle belly) by a researcher until the participant indicates the point at which the pressure has activated muscle soreness (Lau et al., 2013). The PPT relies on the researcher to correctly implement the measure, applying pressure and carefully observing a participant's response (Chesterton et al., 2007). Failure to consistently apply pressure to the same location during subsequent applications, for instance, would make pre- and post-exercise ratings incomparable. Similar to a VAS, PPT ratings are subject to individual characteristics, for example females have been shown to rate less muscle soreness following PPT applications, compared to males (Chesterton et al., 2003). Besides requiring the researchers' careful observation of a participant's response, it also requires accurate reporting by the participant. As such, if the point at which the level of pressure has activated muscle soreness is not properly communicated, it may compromise the validity and reliability of this measure. Moreover, the magnitude of muscle soreness and timeline of recovery using PPT poorly correlates to the commonly used VAS (Clifford et al., 2019); although researchers suggest that they both quantify muscle soreness in different ways (Lau et al., 2013).

2.2.4.2 Exercise Performance

Various tests of exercise performance are also used to assess before and after changes in muscular function following EIMD (Table 2.2). Among those sometimes used by researchers are the countermovement jump (CMJ), isometric midthigh pull (IMTP), maximal voluntary isometric contraction (MVIC), and running economy test. The CMJ is maximal test of lower body power that is frequently used in research (Table 2.2). Using a force plate or digital jump mat to measure ground reaction force (N) or flight time (s), participants perform the jump by first rapidly descending into a 90° squat, and then straight into a maximal vertical jump—all while keeping the hands on the hips and ensuring the knees do not tuck when in the air (Aben et al., 2020). The CMJ is an eccentric-concentric movement that produces force from the stretch-shortening cycle and the release of elastic energy stored in the muscle-tendon unit (Prowting et al., 2021). As such, muscle actions performed during the CMJ may add to its functional relevance as a measure of exercise performance in muscles that have been eccentrically damaged. The CMJ is a sport-specific movement that has been shown to be a reliable test of exercise performance (Hilkens et al., 2021).

It can also be used to monitor central fatigue following EIMD, due to jump height being an accumulative product of maximal force, the rate of force development, and neural functioning (Lombard et al., 2017). However, as an immediate decline in jump height predominantly reflects central fatigue (Knihs et al., 2022), the CMJ's use soon after EIMD may overestimate any ultrastructural muscle damage. Additionally, it requires the activation and synchronisation of multiple muscles, which may increase the risk of injury when having to perform it soon after EIMD (Merrigan et al., 2020).

Another exercise performance measure used to by researchers to monitor changes in muscular function is the IMTP (Merrigan et al., 2021). Traditionally, the IMTP requires a force plate that analyses ground reaction force to determine peak isometric force (N) and rate of force development (N/sec) of the hip and knee extensors during a pull of an immovable object (Grgic et al., 2022). However, force plates are expensive (Couto et al., 2023), therefore a more feasible option of comparable reliability and validity, is a load cell and a custom-made platform that measures peak isometric force in kilograms (Till et al., 2018). To begin the measure, participants start by standing on the force plate or custom-made platform in a position resembling the second pull in the clean Olympic lift (Couto et al., 2023; Halperin et al., 2016). After a verbal countdown, participants then pull the immoveable bar or handle (situated at midthigh level) with maximal effort for a number of seconds. Following a short rest, it is then repeated several times to increase its validity (Aben et al., 2020). The IMTP is a useful measure of maximal force that does not come with great injury-risk (Grgic et al., 2022). Furthermore, as an alternative to one repetition maximum (1RM) testing, it is less fatiguing and more time efficient to perform (Giles et al., 2022). It is also positively correlated with many dynamic lifts/movements, even when no displacement of the body occurs (Merrigan et al., 2020). However, participants have displayed a learning effect while performing the IMPT (Grgic et al., 2022)—meaning that performance could improve over time through practice and repeated exposure, and not due to the effect of an intervention. Therefore, a thorough familiarisation is required, as with all EIMD measures. Moreover, the use of custom-made platforms is not highly standardised, where slight differences in equipment, setups, and/or procedures may alter the replicability of the IMTP (Till et al., 2018).

Corresponding to the IMTP, the MVIC is also a test of isometric force used to monitor changes in muscular function following EIMD (Morton et al., 2005). It is a widely used measure of exercise performance that is highly standardised and reliable (Meldrum et al., 2003). The MVIC can be performed using a dynamometer (e.g., hand-held device, strain gauge, or Biodex/similar

machine) that measures peak isometric force (N) and rate of force development (N/sec) during a maximal contraction of the limb against an immovable object or fixed lever arm set at a certain angle, while a participant is secured in an upright or supine position (Morton et al., 2005). When compared to other measures of exercise performance, it is generally viewed as the gold standard of tests based on its higher feasibility and safety over 1RM testing (Morton et al., 2005). However, to maintain its reliability, unfamiliar researchers and/or participants must be familiarised prior to its usage (Meldrum et al., 2003). It is also important to consider the timing of when this measure is used, as isometric force is the sum of multiple factors, including neurological functioning (Morton et al., 2005). Therefore, it may not be a useful measure of force loss during or immediately after exercise unless paired with electromyography to account for central fatigue (Peñailillo et al., 2015). Lastly, despite the feasibility, safety, and reliability of the MVIC, the Biodex or other similar dynamometers that use a computer can be costly pieces of equipment, which also require a level of expertise to use correctly (Khaitin et al., 2021).

Different to the above measures that can be used to assess power or force output, running economy assesses cardiovascular function following EIMD (Burgess & Lambert, 2010). Running economy can be defined as the oxygen efficiency (L/min) or the metabolic cost of running at a chosen submaximal running intensity (Burt et al., 2023). It is measured using a breath-by-breath metabolic gas analyser that calculates oxygen consumption (L/min) while a participant runs on a motorised treadmill at a selected intensity of their previously determined VO_2max speed (Marcora & Bosio, 2006). Although running economy tests are high in ecological validity and allow exercise performance to be measured without the need for maximal effort, performance is not affected immediately or soon after EIMD (Burt et al., 2023). It is only after DOMS sets in 24 to 48 hours post-EIMD, that normal gait pattern changes due to decrease in stride length and an increase in stride frequency. Consequently, as the body becomes less economically efficient and oxygen consumption increases, running economy then reduces (Vassilis et al., 2008). Therefore, it is only a valid test of exercise performance in the days following EIMD (Burt et al., 2023). Furthermore, previous studies using running economy to measure the impact of EIMD may not necessarily test it at the same running intensity, which reduces this measures' comparability between different studies (Burt et al., 2023). Lastly, the timing of testing, familiarity of treadmill running, and the type of footwear can also affect the results of running economy tests (Saunders et al., 2004).

2.2.4.3 Biomarker Analysis

Lastly, biomarker analysis is often used concurrently to increase the validity and reliability of other indirect measures of EIMD (Chalchat et al., 2022). From a sample of blood (usually collected using venepuncture) that has been centrifuged down and separated into serum or plasma, the concentration of biomarkers is analysed to assess muscle damage and inflammation (McKune et al., 2011). This approach is more objective than measures of muscle soreness and less fatiguing than exercise performance tests (Haller et al., 2023). It is also less invasive and requires less specialist training than muscle biopsies (Etheridge et al., 2008). However, blood collection still requires a trained phlebotomist; although a small droplet of blood can be collected using capillary sampling with a lancet device to puncture the skin (Haller et al., 2023). Additionally, biomarkers only represent the systemic response, not specific localised effects (Baird et al., 2012), and they do not accurately reflect the magnitude of EIMD (Chalchat et al., 2022). They may also indicate changes unrelated to EIMD, including an acute illness or simply an increase due to exercise itself (even when EIMD is not induced; Haller et al., 2023). Lastly, biomarker responses also have large inter-individual variability, with abnormally low or high responses potentially reducing the validity of analysis (Baird et al., 2012).

2.3 Nutritional Interventions

EIMD is an acute ultrastructural muscular injury that, in most cases, resolves naturally over time (Kanda et al., 2013). However, it has been shown to impair exercise performance during subsequent bouts of exercise (Hody et al., 2019) and/or cause a disruption to normal daily activities that are otherwise easy to perform without DOMS (Tesarz et al., 2012). Consequently, these problems may warrant a need for nutritional intervention. Relevant to this thesis, two supplements used to expedite the recovery from EIMD are dairy protein (Master & Macedo, 2021), and to a lesser extent, collagen (Holwerda & van Loon, 2022).

2.3.1 Dairy Protein

The consumption of dairy protein has recently increased in popularity, where the industry has experienced rapid economic growth over recent years (Patel et al., 2023). Successful marketing campaigns often emphasise its ability to promote muscle growth and recovery when

combined with resistance exercise, which has contributed to its popularity (Master & Macedo, 2021). As a supplement, dairy protein is widely available in practical ready-to-drink options, bars, or powdered forms that can be added to water or milk. It is a complete source of protein that scores high on amino acid digestibility and bioavailability indices, as it contains all the essential amino acids that cannot otherwise be endogenously synthesised (McLain et al., 2015).

Whey is the liquid constituent found in whole milk protein and is produced using ultrafiltration to separate it from the milk solids (Master & Macedo, 2021). This process yields 20% whey protein that, after further processing, can be made into concentrated, isolated, and hydrolysed forms with different nutritional profiles (McLain et al., 2015). Once a discarded waste by-product of cheese manufacturing, whey protein has become a valuable product that is among the most popular post-exercise supplements on the market (Auestad & Layman, 2021). One reason for its rise in popularity is its favourable nutritional profile. Whey protein is a highly soluble, fast-digesting, and fast-absorbing protein that causes a rapid increase in postprandial amino acid concentrations and muscle protein synthesis (Auestad & Layman, 2021). Moreover, it has the highest overall essential amino acid content, and is particularly high in cysteine (an essential amino acid with antioxidative effects; Patel et al., 2024) and leucine (an essential amino acid with anabolic effects; Table 2.3; McLain et al., 2015). Whey also contains individual proteins β-lactoglobulin, α-lactalbumin, glycomacropeptide, immunoglobulin, lactoferrin, and serum albumin that have the potential to exert bioactive effects on the human body (e.g., reduce inflammation; Master & Macedo, 2021). However, there are higher economic costs involved in the processing of whole milk protein in order to yield a small percentage of whey protein (Mitchell et al., 2015). Processing also creates by-products that do not have any valuable or substantial use (Zandona et al., 2021). Additionally, the majority of hydrolysed whey proteins have a bitter taste and do not easily dissolve in water (Newman et al., 2015; Uluko et al., 2016).

Yielding a larger percentage after processing (80%), casein protein is the other constituent of the solid portion found in whole milk protein (Auestad & Layman, 2021). Similar to whey protein, it can be further processed or separated to produce concentrated or isolated forms that have differing nutritional properties (Wang et al., 2013). Casein protein is non-soluble, slow digesting, and slow absorbing, and contains individual αs1, αs2, β- casein, and κ-casein proteins (Auestad & Layman, 2021). It provides lower but more sustained postprandial amino acid concentrations and rates of muscle protein synthesis over an extended period of time, and as such, it is often used as a pre-sleep supplement (Master & Macedo, 2021). Furthermore, the

digestive properties of casein protein may increase feelings of fullness; although on the same note, they may also cause bloating and gastrointestinal discomfort due to curdling in the stomach (Auestad & Layman, 2021). Moreover, casein protein has a smaller proportion of essential amino acids, particularly containing less leucine (Table 2.3). Therefore, a higher dose is required to achieve similar postprandial amino acid concentrations and rates of muscle protein synthesis as whey protein (Auestad & Layman, 2021).

Alternatively, whole milk protein, extracted and concentrated from raw skimmed milk through processing (e.g., ultrafiltration, spray drying etc.), offers both benefits in their naturally occurring protein proportions, without the extra economic cost of additional processing (Auestad & Layman, 2021). According to Lacroix et al. (2006), the consumption of whole milk protein has a synergistic effect, where simultaneously there is an immediate effect from faster-acting whey protein and also a sustained effect from slower-acting casein protein. Nonetheless, despite these effects, much of the research on nutritional interventions and exercise recovery has focused on its separated constituents, most notably whey protein (Auestad & Layman, 2021; Master & Macedo, 2021; McLain et al., 2015). However, it is possible that there is less focus on whole milk protein because it takes longer to digest (compared to whey protein) due to the longer chains of amino acids, which is not ideal for those seeking a fast-acting exercise recovery strategy. Whole milk protein is also generally higher in lactose (an issue for those who are lactose intolerant) and may contain other allergens that are removed or reduced during further stages of processing (Abd El-Salam & EI-Shibiny, 2021).

Table 2.3

Nutritional Profile of Dairy Protein

Component	Whole Milk	Casein	Whey		
			Concentrate	Isolate	Hydrolysate
Protein (%)	~80%	~90%	~25-89%	~90-95%	> 90%
Fat (%)	~1.5%	< 1%	1-9%	< 1%	< 1%
Lactose (%)	~5%	0.1-1.5%	4-21%	< 1%	< 1%
Essential AA content (g/100g)	45-49.3	48.9		63-66	
PDCAA score	1.21	1.23		1.15	
Leucine content (g/100g)	7.0	5.8		8.6	

Note. Information sourced from Auestad and Layman (2021), Božanić et al. (2014), Draganidis et al. (2017), Holwerda and van Loon (2022), Master and Macedo (2021), and McLain et al. (2015). Amino acid (AA), Protein Digestibility-Corrected Amino Acid Score (PDCCA).

2.3.1.1 Effect of Dairy Protein on Markers of EIMD

The consumption of dairy protein has been demonstrated to benefit recovery for several markers of EIMD (Table 2.4). Previous research has suggested that it may attenuate DOMS (Cockburn et al., 2010), improve the recovery of muscular function (Cooke et al., 2010), and minimise muscle damage and inflammation, reflected by a reduction in biomarkers during the acute recovery period (Nieman et al., 2020). However, the effect of dairy protein on recovery from EIMD has been inconsistent, despite the large number of studies.

Table 2.4

Effect of Dairy Protein on Markers of EIMD

			Biomarker(s)	
Author(s)	Muscle Soreness	Muscular Function	Muscle Damage	Inflammation
Aussieker et al. (2023)	No	-	-	-
Apweiler et al. (2019)	No	No	-	-
Betts et al. (2009)	No	No	No	No
Brown et al. (2018)	No	Yes	Yes	-
Buckley et al. (2010)	No	Yes	No	No
Cockburn et al. (2008)	No	Yes	Yes	-
Cockburn et al. (2010)	Yes	Yes	Yes	-
Cooke et al. (2010)	-	Yes	No	-
Dahlstrom Burnley et al. (2010)	No	No	No	-
Davies et al. (2020)	No	No	No	-
Draganidis et al. (2017)	Yes	Yes	No	No
Eddens et al. (2017)	No	No	No	No
Etheridge et al. (2008)	No	Yes	No	-
Gee et al. (2019)	No	No	-	-
Hilkens et al. (2021)	No	No	No	No
Hirose et al. (2013)	Yes	-	Yes	-
Nieman et al. (2020)	No	No	Yes	No
Ormsbee et al. (2022)	No	No	No	No
Saracino et al. (2020)	No	No	No	No
ten Haaf et al. (2021)	No	-	No	-
West et al. (2017)	-	Yes	-	-

White et al. (2008)	No	No	No	-

Dairy protein supplementation has been shown to minimise DOMS following EIMD (Table 2.4; Cockburn et al., 2010). For instance, milk protein concentrate drinks (4 x 20 g on EIMD day & 20 g· day for 8 days), consumed by trained males after 300 unilateral eccentric knee contractions, reduced post-exercise muscle soreness (based on ratings from a continuous-range scale) better than an isoenergetic carbohydrate (CHO) PLA in a 6-week crossover study (Draganidis et al., 2017). Moreover, in another crossover study, milk protein (5 g of before and 5 g after EIMD, then 10 g 2x daily for 8 days) attenuated self-reported muscle soreness that was induced by eccentric calf raises in untrained males, whereas those in control condition (CON) group that consumed nothing experienced no such benefit (Hirose et al., 2013).

Although the studies above suggest that dairy protein alleviates DOMS, the majority of research indicates that this is probably not the case (Table 2.4; Apweiler et al., 2019; Aussieker et al., 2023; Betts et al., 2009; Brown et al., 2018; Buckley et al., 2010; Cockburn et al., 2008; Dahlstrom Burnley et al., 2010; Davies et al., 2020; Eddens et al., 2017; Etheridge et al., 2008; Gee et al., 2019; Hilkens et al., 2021; Nieman et al., 2020; Ormsbee et al., 2022; Saracino et al., 2020; White et al., 2008). For example, Gee et al. (2019) did not observe a reduction in DOMS, based on ratings from VAS, when resistance trained males consumed 39 g of whey protein or an isoenergetic CHO PLA post-EIMD for three days after resistance exercise. Whey protein (0.33 g· kg· day) also had no effect on DOMS, when it was consumed daily after exercise over a 7-day period of resistance training, in resistance trained males (Davies et al., 2020). Moreover, an isoenergic CHO PLA proved more effective at reducing DOMS, compared to one 250 mL serve of milk protein consumed daily for three days, following a 15-km run in recreationally active participants (ten Haaf et al., 2021).

Alternatively, dairy protein supplementation may have more success at improving the recovery of muscular function (Table 2.4; Brown et al., 2018; Cockburn et al., 2010). For instance, Etheridge et al. (2008) found a single 100 g dose of milk protein (post-EIMD) increased the recovery of isometric force and peak power output at 48 hours post-exercise, compared to a flavoured water PLA, in recreationally active males that completed a 30-minute downhill. Furthermore, two 500 mL serves of a commercially available chocolate milkshake, containing milk protein (33.4 g of protein) and CHO (118.2 g), consumed by trained males immediately following and two hours after unilateral eccentric-concentric actions of the knee flexors, attenuated

isokinetic torque 48 hours post-EIMD in a study by Cockburn et al. (2008). Additionally, Cooke et al. (2010) found that whey protein isolate supplementation (1.5 g· kg· day) for 14 days post-EIMD, improved the recovery of isometric and isokinetic force in untrained males following a bout of resistance exercise. Buckley et al. (2010) also found that a single dose of a hydrolysate of whey protein isolate (25 g post-EIMD) was beneficial for the recovery of isometric force following eccentric-exercise in sedentary males. Lastly, whey protein (25 g· day), consumed immediately following resistance exercise and the morning after, was more effective than an energy-matched PLA at attenuating isometric force, CMJ, and peak power output loss in trained males; although, it had no effect on recovery of repetitions to failure performance (West et al., 2017).

Conversely, numerous studies have also failed to observe an effect of dairy protein supplementation on recovery of muscular function following EIMD (Betts et al., 2009; Gee et al., 2019; Nieman et al., 2020; Ormsbee et al., 2020; Saracino et al., 2020; White et al., 2008). For instance, a single 40 g pre-sleep bolus of casein protein did not improve recovery of CMJ and isometric force performance at 24 and 48 hours after 100 drop jumps that were performed in the morning by recreationally active males and females (Apweiiler et al., 2019). Moreover, 0.4 g· kg of whey protein (post-EIMD for 3 days) failed to attenuate muscular function during a number of exercise tests better than an iso-energetic PLA after untrained males performed eccentric-exercise in a crossover study (Dahlstrom Burnley et al., 2010). Hilkens et al. (2021) also found that 30 g of whey protein, twice a day over a 9-day period (both pre- & post-EIMD), had no effect on recovery of CMJ and isometric force performance after recreationally active males performed 100 drop jumps, and that an isoenergetic CHO PLA was more effective at recovering isokinetic torque**.** Additionally, whey protein concentrate (0.33 g· kg· day) that was consumed each morning during a 7-day resistance exercise protocol had no effect on CMJ or isometric force performance, compared to an isonitrogenous formulation of non-essential amino acids, in resistance-trained males (Davies et al., 2020). Finally, Eddens et al. (2017) found two 20 g doses of whey protein hydrolysate, consumed daily for four days post-EIMD, had no effect on the CMJ and isometric force performance of well-trained cyclists after they performed simulated high intensity road cycling and 100 drop jumps.

On the other hand, the consumption of dairy protein may have a positive effect on muscle damage, reflected by a reduction in muscle damage biomarkers following EIMD (Table 2.4; Cockburn et al., 2010; Hirose et al., 2013). Cockburn et al. (2010) found that 1000 mL of a ready-to-drink milk protein (33.4 g protein) and CHO (118.2 g of CHO) supplement, consumed before

exercise, attenuated CK 48 hours after unilateral eccentric-concentric knee flexions performed by trained males. Furthermore, two doses of whey protein hydrolysate (70 mL), taken for four days post-EIMD, also attenuated the increase in CK at 48 hours after females completed a protocol of repeated-sprints (15 x 30 m sprints with a rapid 10 m deceleration phase; Brown et al., 2018). Nieman et al. (2020) also observed a positive effect on muscle damage biomarkers, reporting that 0.3 g· kg of whey protein taken pre- and post-exercise, and before sleep, over a 5-day period, attenuated CK and Mb at day four and five after a 90-min eccentric exercise bout performed by non-active males.

Although some previous studies have reported a positive effect, most have found no attenuation in biomarkers of muscle damage after intervening in recovery from EIMD with dairy protein (Betts et al., 2009; Buckley et al., 2010; Dahlstrom Burnley et al., 2010; Cooke et al., 2010; Davies et al., 2020; Eddens et al., 2017; Etheridge et al., 2008; Hilkens et al., 2021; Ormsbee at al., 2022; White et al., 2008). For example, Saracino et al. (2020) found that a single 40 g dose of whey protein hydrolysate or whey protein isolate, consumed 30 minutes before sleep, had no effect on CK following maximal eccentric knee contractions performed in the morning in recreational males. Ormsbee et al. (2022) also failed to observe an attenuation in CK at 12 hours post-exercise, when resistance trained participants immediately consumed a single 40 g dose of pre-sleep casein after completing a bout of resistance exercise. In addition, ten Haaf et al. (2021) also found 250 mL of milk protein, consumed daily for three days, did not reduce CK or LDH following a 15-km race in recreationally active runners (ten Haaf et al., 2021).

Given the potential of dairy protein to exert a benefit on other markers of EIMD, it might be reasonable to suggest that it may also reduce inflammation. Yet, among previous studies that have measured exercise-induced inflammation, most have failed to observe a benefit (Table 2.4; Draganidis et al., 2017; Eddens et al., 2017; Hilkens et al., 2021; Nieman et al., 2020; Ormsbee et al., 2022; Saracino et al., 2020). For example, whey protein isolate combined with CHO (0.4 g· kg· hour for 4 hours), consumed after 90 minutes of shuttle running, had no effect on IL-6, IL-10, and CRP in highly trained males; although CRP did not significantly increase above baseline (Betts et al., 2009). Moreover, a single 25 g serve of hydrolysed whey protein isolate or whey protein isolate (post-EIMD) also had no effect on the inflammatory biomarker TNFα following 100 maximal eccentric knee extensions in sedentary males (Buckley et al., 2010). However, in the same study, TNFα did not significantly increase above baseline after EIMD.

Despite having reported some potential benefits after dairy protein supplementation, previous studies that have examined its effect on acute recovery from EIMD have produced inconsistent findings. This could be influenced by a number of limitations, including potential study design flaws, ineffective EIMD protocols, and a possible disregard for a participants' training history; these will be outlined in more detail in Section 2.4.

2.3.1.2 Mechanistic Actions of Dairy Protein

Several different mechanisms have been suggested to be responsible for the effect of dairy protein on recovery from EIMD. The main mechanism of action is believed to be the positive effect supplementation has on protein metabolism (Hirose et al., 2013). Dairy protein provides essential amino acids that cannot otherwise be synthesised endogenously, which are then used to create new muscle proteins (Reitelseder et al., 2014). This consequential increase in amino acid availability following supplementation is thought to enhance rates of muscle protein synthesis, which improves muscle repair after EIMD (Casagranda et al. 2019; West et al., 2017). For example, one 30 g dose of whey protein, consumed immediately after exercise, increased rates of muscle protein synthesis following a bout of resistance exercise in recreational athletes (Aussieker et al., 2023). Additionally, an increase in amino acid availability may also supress the breakdown of muscle proteins (Tipton et al., 1999), and subsequently improve muscle recovery following EIMD (Reitelseder et al., 2014).

Despite existing studies often attributing the benefits of dairy protein to its positive effects on muscle protein synthesis (Aussieker et al., 2023; Brown et al., 2018; Dahlstrom Burnley et al., 2010; ten Haaf et al., 2021), some researchers have disputed whether this is the precise mechanism(s) responsible. According to Pavis et al. (2021), it is possible for dairy protein to benefit recovery without stimulating muscle protein synthesis. In their study, they found that acute postprandial myofibrillar protein synthesis rates (using muscle biopsies) were maximal post-eccentric exercise and were thus unaffected by the consumption of a daily protein-polyphenol drink for seven days, despite supplementation having accelerated muscular recovery after 300 eccentric knee contractions in recreationally active participants. Therefore, it is likely that dairy protein may benefit recovery from EIMD through other muscle repair processes.

Dairy protein supplementation may increase the activity of satellite cells, muscle stem cells that regulate muscle growth and regeneration, and support the proteasome pathway, an

enzymatic process that maintains myocellular homeostasis (Draganidis et al., 2017). However, as mentioned above, it is difficult to find evidence for alternative muscle repair processes, because most research has positioned muscle protein synthesis as the main mechanism of action (Aussieker et al., 2023; Brown et al., 2018; Dahlstrom Burnley et al., 2010; ten Haaf et al., 2021).

'Bioactive' or hydrolysed forms of dairy protein, such as whey, may also have a positive effect on recovery by minimising exercise-induced inflammation and oxidative stress (Auestad & Layman, 2021). Consumption has been suggested to inhibit the release of cytokines, leukocytes, enzymes, and other signalling pathways that amplify the inflammatory response after exercise (Da Silva et al., 2017; Draganidis et al., 2017). However, alongside unaffected rates of postprandial myofibrillar protein synthesis, Pavis et al. (2021) also found that pro-inflammatory signalling pathways were unaffected by the protein-polyphenol drink. Alternatively, immune cells also produce harmful by-products that supplementation may neutralise. According to Peng et al. (2009), whey protein isolate has strong anti-oxidant and free radical scavenging activity. In the context of EIMD, a whey protein isolate drink, consumed daily for eight days, attenuated oxidative stress biomarkers after 45 minutes of exercise in trained males cyclists (Ton et al., 2017).

The consumption of dairy protein may also benefit recovery by improving insulinemic and glycaemic responses following EIMD. Although not in the context of EIMD, Morifuji et al. (2010) found that 12.5 g of whey protein hydrolysates without CHO stimulated the release of insulin following ingestion. Furthermore, according to Betts and Williams (2010), dairy protein supplementation (when co-ingested with CHO) may increase rates of muscle glycogen resynthesis, replenishing depleted stores after exercise.

Dairy protein supplementation is believed to exert a range of mechanistic actions on the body following EIMD. From the delivery of essential amino acids that are thought to stimulate rates of muscle protein synthesis and prevent muscle protein breakdown, to a reduction in inflammation and oxidative stress from bioactive properties, and an improvement in cellular metabolism. Each of these actions, in turn, may work to minimise markers and expedite recovery from EIMD.

2.3.2 Collagen

Collagen is a fibrous protein that contributes to 25 to 30% of the total protein found within the human body (Holwerda & van Loon, 2022). Large quantities are located within the

musculoskeletal system, where together it forms 80% of the dry mass in tendons and ligaments (Shenoy et al., 2022). Within connective tissue, collagen crosslinks join to create a lattice type structure that provides the strength and elasticity required to withstand the tensile and compressive forces of the musculoskeletal system (Hashim et al., 2015). There are over 29 different collagen subtypes that are appropriately categorised by their functional properties and fibril producing ability (Shenoy et al., 2022). Among these, type I, II, III, and IV are the most abundant in muscles and bones (Gelse et al., 2003). Each type is comprised of three alpha chains that hold ~1000 amino acid residues, which are arranged into a right-handed triple helix structure (Shenoy et al., 2022). Relative to the repeated sequencing Gly-X-Y on each chain, the non-essential amino acid glycine (~33%) is the most abundant, occupying every third amino acid position, followed by proline (~10%) and hydroxyproline (~13.5%) that typically occupy the X and Y positions (Holwerda & van Loon, 2022).

As a supplement, collagen can be extracted from the collagen-rich tissues (hide, bones & scales) of different animal sources, including bovine, porcine, marine and poultry (Holwerda & van Loon, 2022). While there are different types of consumable dairy protein (e.g., whey, casein etc.), collagen hydrolysate is perhaps the only type consumed with the intention to improve recovery from EIMD (Aussieker et al., 2023; Bischof et al., 2024; Clifford et al., 2019; Lopez et al., 2015; Prowting et al., 2021; Robberechts et al., 2023). It is a type of collagen that has undergone several stages of processing (e.g., pre-treatment, extraction, and drying) that denatures long chains of amino acids into smaller peptides using three main methods of extraction; enzymatic, microbial or chemical (Xu et al., 2023). This makes it easier for the digestive enzymes of the stomach to break them down further before they can be absorbed by the small intestine and into the bloodstream (Kviatkovsky et al., 2022). Collagen hydrolysates' greater bioavailability and better absorption properties may also allow it to increase post-prandial concentrations of non-essential amino acids in plasma better than unprocessed collagen (Holwerda & van Loon, 2022).

Distinct from dairy protein, collagen is an incomplete source of protein that does not contain a large number of essential amino acids; specifically lacking those that stimulate muscle anabolism (Deane et al., 2020). Furthermore, without the essential amino acid tryptophan, it scores much lower on the Protein Digestibility-Corrected Amino Acid Index (Table 2.5; Phillips, 2016). However, despite being a suboptimal protein, collagen is lactose-free and rich in non-essential amino acids used to synthesise collagen during the remodelling of the extracellular matrix (Holwerda & van Loon, 2022). It is also highly versatile and can be easily incorporated into

various post-exercise beverages, including smoothies (Amyoony et al., 2023). Moreover, there is an ample supply of by-products to source collagen from; a source that might be more sustainable and economically viable than other protein sources (Fu et al., 2019; Xu et al., 2023). However, mammalian sourced collagen carries a risk of disease, along with several cultural or religious concerns that are associated with its consumption (Duasa et al., 2023). Furthermore, marine sourced collagen is harder to incorporate into beverages that do not easily mask its strong fishy flavours and/or aromas (Amyoony et al., 2023). Lastly, collagen is ultimately a by-product of the meat industry, so there could be some preconceived notions that need to be addressed before it gains greater consumer acceptance (Fu et al., 2019)

Table 2.5

Nutritional Profile of Collagen Hydrolysate

Component	Collagen Hydrolysate
Protein (%)	> 90
Fat (%)	< 1
Lactose (%)	0
Essential AA content (g/100g)	4.63
PDCAA score	0
Leucine content (g/100g)	0.79

Note. Information sourced from Aussieker et al. (2023), Hilkens et al. (2023), and Phillips et al. (2016).

2.3.2.1 Effect of Collagen on Markers of EIMD

Although the existing literature on collagen in the context of exercise is limited (Deane et al., 2020), a small number of studies have demonstrated a positive effect on recovery from EIMD (Table 2.6). Supplementation has reduced DOMS (Lopez et al., 2015), improved recovery for various measures of muscular function (Clifford et al., 2019), and attenuated biomarkers of muscle damage and inflammation (Lopez et al. 2015). However, as with the effect of dairy protein, previous findings have also been inconsistent.

Table 2.6

Effect of Collagen on Markers of EIMD

Author(s)	Muscle Soreness	Muscular Function	Biomarker(s)	
			Muscle Damage	Inflammation
Aussieker et al. (2023)	No	-	-	-
Bischof et al. (2024)	-	-	Yes	No
Clifford et al. (2019)	Yes	Yes	No	Yes
Lopez et. al (2015)	Yes	Yes	Yes	Yes
Prowting et al. (2021)	No	Yes	-	-
Robberechts et al. (2023)	No	No	No	No

Consumed around muscle-damaging exercise, collagen may alleviate sensations of DOMS (Table 2.6; Lopez et al., 2015). Clifford et al. (2019) found two 10 g serves of collagen hydrolysate, consumed daily for seven days before and two days post-exercise, had nonsignificant 'possible' (24 hours) and 'likely' (48 hours) benefits on recovery of DOMS after 150 drop jumps in recreationally active males. Additionally, a smaller amount of collagen hydrolysate (1.5 g in the morning and 1.5 g in the evening), consumed over a longer period of six weeks prior to a retest of an upper body resistance exercise, accelerated DOMS recovery in recreationally active adults (Lopez et al., 2015). However, these findings were preliminary, and Lopez et al. (2015) suggest that a larger study is necessary to verify these effects.

In contrast, previous studies have also found that collagen consumption does not reduce exercise-induced DOMS (Robberechts et al., 2023). For example, a single 30 g dose of collagen hydrolysate, consumed immediately following a bout of resistance exercise, did not reduce DOMS better than a non-energetic PLA in young recreational athletes (Aussieker et al., 2023). Likewise, Prowting et al. (2021) also found no effect of collagen hydrolysate (15 g· day), consumed daily for seven days prior and five days after 100 drop jumps, on DOMS recovery in resistance trained males.

Alternatively, collagen supplementation may benefit recovery of muscular function following EIMD (Table 2.6; Clifford et al., 2019). Despite Prowting et al. (2021) having failed to observe a reduction in DOMS, they did, however, observe an improvement in recovery of CMJ performance at 24 hours post-exercise. Clifford et al. (2019) also found an effect on CMJ performance, having reported a faster recovery of jump height at 48 hours after drop jumps in the collagen hydrolysate group. Additionally, Lopez et al. (2015) observed a lower decrease in the number of reps performed during a pre-test (day 43) and re-test (day 46) of an upper body resistance exercise test in the collagen hydrolysate group, compared to the cellulose PLA group

However, not all studies have found an effect of collagen supplementation on muscular function (Table 2.6). Robberechts et al. (2023) found no attenuation, nor a faster recovery in CMJ, isometric force, and dynamic torque performances after young fit males consumed 20 g of collagen hydrolysate combined with 25 g of whey protein before and during a 3-week training program (unilateral eccentric knee extensor exercises). Additionally, all available studies on collagen and recovery from EIMD have failed to observe an overall improvement in all measures of exercise performance. The studies by Clifford et al. (2019) and Prowting et al. (2021) found no effect of collagen hydrolysate on recovery of MVIC performance, despite observing an effect on CMJ performance following EIMD. According to Clifford, this discrepancy was caused by large inter-participant variability in CMJ and MVIC performance that possibly reduced their ability to observe an effect in the latter measure, whereas Prowting attribute discrepant findings to the different muscle actions used in each measure. More specifically, Prowting explain how the MVIC does not utilise elastic force (like a CMJ), further suggesting the mechanisms behind any benefit of collagen predominantly improve muscle actions that use connective tissue and the stretch-shortening cycle to produce force, such as the CMJ.

Based on biomarkers, the consumption of collagen may have an effect on ultrastructural muscle damage after exercise (Table 2.6; Lopez et al., 2015). Next to other reported benefits, the pilot study by Lopez et al. (2015) also found that six weeks of collagen hydrolysate, consumed prior to the retest of an upper body exercise test, attenuated CK and LDH when compared to a PLA. Moreover, in a study using twice the duration of supplementation, 12 weeks of 'specific' collagen peptides (15 g· day), consumed after the first and before the second trial of 150 drop jumps, reduced Mb and LDH in sedentary to moderately active males participating in concurrent exercise. Conversely, Clifford et al. (2019) reported no effect of collagen hydrolysate supplementation on the post-exercise efflux of CK, LDH, aspartate transaminase (AST), and alanine transaminase (ALT). Similarly, Robberechts et al. (2023) also failed to observe a difference in CK between collagen combined with whey protein and whey protein only groups during a 3-week training program.

Finally, the supplementation of collagen may have an effect on exercise-induced inflammation (Table 2.6). For example, Clifford et al. (2019) found a moderate decrease in IL-6 at 1.5 hours post-exercise in the collagen hydrolysate group; although there was no decrease in beta-nerve growth factor (β-NGF) that failed to rise above baseline. Moreover, Lopez et al. (2015) observed a smaller CRP increase after an upper body retest, when participants consumed collagen

hydrolysate. However, in Robberechts et al. (2023) the co-ingestion of collagen hydrolysate and whey protein had no effect on IL-6, but this inflammatory biomarker also failed to increase above baseline after EIMD. Similarly, Bischof et al. (2024) did not observe a positive effect of 'specific' collagen peptides on exercise-induced inflammation based on hsCRP.

Despite some inconsistent effects on markers, the supplementation of collagen might be an effective nutritional intervention for the treatment of EIMD. However, similar to dairy protein, previous research has several limitations, which will be presented in Section 2.4 along with those from dairy protein research. Moreover, the existing literature needs to be expanded in order to establish whether collagen may indeed benefit recovery from EIMD.

2.3.2.2 Mechanistic Actions of Collagen

The mechanistic actions believed to be responsible for the effect of dairy protein have been well researched, but less is known about the actions of collagen in the context of EIMD (Clifford et al., 2019; Prowting et al., 2021). While dairy protein predominantly aids muscle repair, collagen supplementation may enhance the remodelling of connective tissue (Aussieker et al., 2023). From the extra provision of non-essential amino acids glycine, proline, and hydroxyproline, the consumption of collagen is thought to increase their availability and promote collagen synthesis—a crucial process involved in the replacement of damaged or worn-out collagen fibrils within the extracellular matrix (Alcock et al., 2019). Collagen may also increase the enzymatic activity of matrix metalloproteinases, enzymes that clear collagen debris and maintain the healthy turnover of collagen (Kviatkovsky et al., 2022). Shaw et al. (2016) reported 15 g of collagen (vitamin-C enriched gelatin), consumed by healthy males after a short skipping exercise, increased plasma glycine, proline, and hydroxyproline, and biomarkers indicating collagen synthesis. However, according to Prowting et al. (2021), the extra provision of non-essential amino acids is not required to increase collagen synthesis, stating that it is possible for the body to sufficiently synthesise them without the need for an exogenous supply. Furthermore, existing studies have also found that collagen supplementation does not enhance collagen synthesis after EIMD (Clifford et al., 2019; Protwing et al., 2021; Robberechts et al., 2023). Even after a ~30-fold increase in post prandial non-essential amino acid concentration from collagen supplementation, Aussieker et al. (2023) found no increase in collagen synthesis. Regardless, it is difficult to accurately measure collagen synthesis without performing muscle biopsies (Kviatkovsky et al., 2022). Moreover,

commonly used biomarkers of collagen synthesis are not specific to the synthesis of collagen in connective tissue, as they also reflect the synthesis of collagen in bone (Prowting et al., 2021).

Combined with collagen synthesis, the consumption of collagen may attenuate the breakdown of collagen in the extracellular matrix following EIMD, providing a type of 'protective' effect against muscle-damaging exercise (Holwerda & van Loon, 2022). For example, Aussieker et al. (2023) found that 30 g of collagen hydrolysate inhibited collagen breakdown in a group of recreational males and female athletes after resistance exercise. However, 20 g of collagen hydrolysate failed to do the same in recreationally active males that performed drop jumps (Clifford et al., 2019). Inconsistent findings may be explained in part by the assessment of collagen breakdown being based on changes in biomarkers. Similar to the synthesis of collagen, biomarkers of breakdown, such as β-CTX predominantly reflect changes that occur in bone (Clifford et al., 2019).

The mechanistic actions of collagen may extend to include a reduction in exercise-induced inflammation (Brandao-Rangel et al., 2022) and oxidative stress (Hao et al., 2023). Hydrolysed collagen contains bioactive peptides that may exert serval anti-inflammatory effects (Paul et al., 2019). Bioactive peptides in hydrolysed marine collagen have been shown to reduce the expression of pro-inflammatory cytokines (Sivaraman & Shanthi, 2021). They may also modulate the inflammatory response by inhibiting the activity of pro-inflammatory cells, enzymes, and pathways that may lead to nociceptor aggravation (Wheeler et al., 1999). However, the effect of collagen on inflammation following EIMD is not clear, and some studies have failed to observe an effect. For example, Robberechts et al. (2023) suggest that the co-ingestion of collagen and whey protein does not reduce inflammation after a 3-week training program.

Alongside a possible effect on the inflammatory response, hydrolysed forms of collagen may also exert antioxidative effects by scavenging and neutralising reactive oxygen species (Hao et al., 2023). León-López et al. (2019) demonstrated how hydrolysed collagen sourced from sheepskins has an antioxidative effect, although the extent of this relied heavily on the level of hydrolysis. However, in the context of oxidative stress induced by exercise, further research is required to specify whether there is any positive effect.

Collagen's mechanisms of action may not be limited to direct positive effects on connective tissue repair/remodelling or by lessening the inflammatory response, it may also have indirect effects on muscle tissue. Supplementation has been shown to indirectly support myofibrillar protein synthesis by maintaining nitrogen balance (Deane 2020). Furthermore, according to

Balshaw et al. (2022), through its direct effects on connective tissue it can also stimulate an increase in myoblast activity, promoting myogenesis and other adaptative responses of the muscle after exercise.

The mechanisms of action through which collagen supplementation may exert a positive effect on recovery from EIMD are believed to be primarily through non-essential amino acids that increase the synthesis of collagen, which assists in the repair of connective tissue following exercise (Prowting et al., 2021). Additionally, but to a lesser extent, collagen supplementation may also minimise exercise-induced inflammation and oxidative stress (Brandao-Rangel et al., 2022; Hao et al., 2023), as well as supporting muscle repair (Balshaw et al., 2022). It may also have other beneficial actions, however more research is needed to understand them in the context of EIMD.

2.3.3 Dosage Variables

2.3.3.1 Protein Type

Different studies have attempted to alter recovery from EIMD using a variety of dairy protein types (Table 2.7). Different types of dairy protein may exert differing effects. For example, whey protein has faster-acting stimulatory effects on rates of muscle protein synthesis, which leads to a higher, yet more transient peak plasma concentration of essential amino acids (Auestad & Layman, 2021). On the other hand, casein protein has slower-acting effects on muscle protein synthesis, which causes a smaller, but sustained increase in essential amino acids in plasma. Whereas milk protein may exert fast- and slow-acting effects on muscle protein synthesis, causing both a higher and more sustained peak concentration of essential amino acids in plasma. Nonetheless, although most studies have intervened with whey protein (Table 2.7), the type selected essentially depends on the specific aims of each study.

Table 2.7

Dairy Protein Dosage Information

Author(s)	Type	Amount Per Serve	Daily Protein Intake*	PLA or CON Type	Timing	Duration	Dietary Control
Apweiler et al. (2019)	CP	40 g	~1.5 g· kg	Non-isoenergetic CHO	Post-EIMD	1 day	Diet record (habitual & during study) & WP/CHO

Author(s)	Type	Amount Per Serve	Daily Protein Intake*	PLA or CON Type	Timing	Duration	Dietary Control
							drink
Aussieker et al. (2023)	WP isolate	30 g	1.3-1.6 g· kg	Flavoured water	Post-EIMD	1 day	Diet record (habitual only)
Betts et al. (2009)	WP isolate (with CHO)	0.4 g kg	-	Non-isoenergetic CHO	Pre- & post-EIMD	1 day	Diet record (habitual & during study)
Brown et al. (2018)	WP hydrolysate	70mL	~1.3 g· kg	Isoenergetic CHO	Post-EIMD	4 days	Catered meals
Buckley et al. (2010)	WP isolate or hydrolysed WP isolate	25 g	-	Flavoured water	Post-EIMD	1 day	-
Cockburn et al. (2008)	MP (with CHO)	500 mL	-	Non-isoenergetic CHO	Post-EIMD	1 day	-
Cockburn et al. (2010)	MP (with CHO)	1000 mL	-	Water	Pre- or post-EIMD	1 day	Diet record (habitual & during study)
Cooke et al. (2010)	Hydrolysed WP isolate (with CHO)	30 g	~0.8 g· kg	Isoenergetic CHO	Post-EIMD	14 days	Diet record (habitual & during study)
Dahlstrom Burnley et al. (2010)	WP	0.4 g· kg	78-83 g	Isoenergetic CHO or flavoured water	Post-EIMD	1 day	Diet record (during study only)
Davies et al. (2020)	WP concentrate	0.33 g· kg	1.7 g· kg	Non-isoenergetic amino acids	Pre-EIMD	6 days	Diet record (habitual only) & catered meals
Draganidis et al. (2017)	MP concentrate	20 g	1-1.2 g· kg	Isoenergetic CHO	Post-EIMD	8 days	Diet recall (habitual only) & diet plan (during study only)
Eddens et al. (2017)	WP hydrolysate	20 g	1.2 g· kg	Isoenergetic CHO or non-energetic PLA	Post-EIMD	4 days	Catered meals
Etheridge et al. (2008)	MP concentrate	100 g	-	Flavoured water	Post-EIMD	1 day	Diet record (during study only)
Gee et al. (2019)	WP hydrolysate (with CHO)	39 g	-	Isoenergetic CHO	Post-EIMD	1 day	Diet record (habitual & during study)
Hilkens et al. (2021)	WP concentrate	25 g/50 g	0.9-1.2 g· kg	Isoenergetic CHO	Pre- & post-EIMD	9 days	Diet recall (habitual & during study)
Hirose et al. (2013)	MP	5 g	-	-	Pre- & post-EIMD	5 days	-
Nieman et al. (2020)	WP isolate	0.3 g· kg	-	Water	Post-EIMD	5 days	-
Ormsbee et al. (2022)	CP	40 g	1.7-1.9 g· kg	Non-energetic PLA	Post-EIMD	1 day	Diet record (habitual & during

Author(s)	Type	Amount Per Serve	Daily Protein Intake*	PLA or CON Type	Timing	Duration	Dietary Control
							study)
Robberechts et al. (2023)	WP isolate	15 g before & 30 g after	~1.2 g· kg	-	Pre- & post-EIMD	4 weeks	Diet record (during study)
Saracino et al. (2020)	WP hydrolysate or WP isolate	40 g	1.1 g· kg	Non-energetic PLA	Post-EIMD	3 days	Catered meals
ten Haaf et al. (2021)	MP	20 g	1.1 g· kg	Isoenergetic CHO	Post-EIMD	4 days	Diet recall (habitual & during study)
West et al. (2017)	WP	25 g	1.9 g· kg	Isoenergetic CHO	Post-EIMD	2 days	Diet record (habitual only) & catered meals
White et al. (2008)	WP (with CHO)	23 g	-	Flavoured water	Pre- or post EIMD	1 day	Post-exercise liquid meal

Note. * Daily protein intake excluding the supplement. Placebo (PLA), control (CON), casein protein (CP) milk protein (MP), carbohydrate (CHO), exercise-induced muscle damage (EIMD).

Similarly, different collagen types may have differing effects on recovery from EIMD. In terms of supplementation, typically there are only two types of collagen that are consumed—gelatin or collagen hydrolysate. Native or unprocessed collagen is usually not one of these, because its outer layer is impenetrable to the digestive enzymes of the stomach (Holwerda & van Loon, 2022). While both gelatin and collagen hydrolysate are more bioavailable than native collagen, collagen hydrolysate might be the most effective. Collagen hydrolysate has undergone a level of processing that enables it to cause a faster postprandial increase in non-essential amino acids (Shenoy et al., 2022), which explains why it is the only form used in the context of EIMD (Table 2.8).

Table 2.8

Collagen Dosage Information

Author(s)	Type	Amount Per Serve	Daily Protein Intake*	PLA or CON Type	Timing	Duration	Dietary Control
Aussieker et al. (2023)	CH	30 g	1.3-1.6 g· kg	Flavoured water	Post-EIMD	1 day	Diet record (habitual)
Bischof et al. (2024)	Specific collagen	15 g	-	Silicea PLA	Pre- & post EIMD	12 weeks	Diet record (during study) &

	peptides						pre-EIMD meal
Clifford et al. (2019)	CH (+ Ribena Light)	10 g	~1.2 g· kg	Isoenergetic CHO	7 days pre- & 2 days post-EIMD	9 days	Diet recall (habitual) & record (during study)
Lopez et. (2015)	CH	1.5 g	-	Non-energetic cellulose	Pre-EIMD	6 weeks	Diet record (habitual & during study)
Prowting et al. (2021)	CH	15 g	~2 g· kg	Isoenergetic CHO	7 days pre- & 5 days post-EIMD	12 days	Diet record (habitual & during study)
Robberechts et al. (2023)	CH (with WP isolate)	10 g CH (+ 5 g WP isolate) before & 10 g CH (+ 20 g WP isolate) after	~1.2 g· kg	-	Pre- & post-EIMD	4 weeks	Diet record (during study)

Note. * Daily protein intake excluding the supplement. Collagen hydrolysate (CH), placebo (PLA), control (CON), exercise-induced muscle damage (EIMD), carbohydrate (CHO), whey protein (WP).

2.3.3.2 Co-ingestion with Other Nutrients

The effect of dairy protein might be enhanced through co-ingestion with CHO (Betts & Williams, 2010). Previously, of the six studies that have combined dairy protein with CHO (Table 2.7), three of these have improved recovery from EIMD following co-ingestion (Cooke et al., 2010; Cockburn et al., 2008; Cockburn et al., 2010). While Howatson et al. (2008) highlight the benefits of co-ingestion in a review (e.g., improves amino acid uptake), it may make it harder to discern whether an improvement is due to dairy protein or the addition of CHO; particularly when no isoenergetic CHO PLA is used to account for the effects of CHO (Cockburn et al., 2008; Cockburn et al., 2010). Admittedly, Cockburn et al. (2010) found it difficult to decipher whether it was the actions of dairy protein or of CHO that improved muscular function recovery following EIMD. Considering these problems, Eddens et al. (2017) choose not to add CHO to dairy protein in order to make it easier for them to establish the cause of any ergogenic effects. Moreover, according to Miller et al. (2003) the combined effect of dairy protein and CHO co-ingestion on recovery mechanisms following exercise is almost equal to the effect of each, when they are ingested alone. Therefore, although co-ingestion might be a means to enhance the overall effect, in doing so, it may make it more challenging for researchers to understand the underlying mechanisms that are responsible, most notably if no isoenergetic CHO PLA is used.

Dairy protein is not the only supplement to have had other nutrients added to it for the purpose of enhancing its overall effect. Some researchers suggest that adding vitamin C to collagen may improve collagen synthesis (Shaw et al., 2016). Certainly, Clifford et al. (2019) reported a benefit to recovery after adding 80 mg of vitamin C in the form of Ribena Light to collagen hydrolysate (Table 2.8). However, Clifford found no increase in collagen synthesis with supplementation. Furthermore, Robberechts et al. (2023) highlight the controversy around whether vitamin C supplementation is, in fact, beneficial for individuals who are not deficient. Additionally, similar to the issues regarding dairy protein and CHO co-ingestion, without a PLA group that does not consume vitamin C, it is difficult to attribute any effects directly to collagen (Robberechts et al., 2023). Nonetheless, Prowting et al. (2021) assert that it still warrants further investigation.

2.3.3.3 Protein Amount Per Serve

The effect of dairy protein on EIMD might also be subject to the amount of protein consumed per serve. The amount consumed in previous studies ranges from 5 to 100 g per serve (Table 2.7). The optimal amount of dairy protein, according to a review by Master and Macedo (2020), is 20 to 40 g. They also suggest that amounts below this will not be enough to maximise recovery processes and anything above will likely cause a muscle-full effect, a point at which the muscle cell reaches its maximum capacity for amino acids and there is no further increase in muscle protein synthesis. Master and Macedo based this suggestion on findings by Witard et al. (2014), who found that 10 g of whey protein was not enough to increase rates of muscle protein synthesis, as it had the same effect as a non-protein PLA, and a larger 40 g dose had the same effect on muscle protein synthesis as a smaller 20 g dose. Therefore, previous studies on dairy protein and EIMD that supplemented under 10 g may have not been enough to see a benefit, and doses that were larger than 40 g may have had no further benefit.

Similarly, the effectiveness of collagen may also be dictated by the amount consumed per serve (Shaw et al., 2016). The amount used to treat EIMD experimentally is between 1.5 to 30 g per serve (Table 2.8). While smaller amounts of collagen have been shown to be effective (Lopez et al., 2015), the amount required to improve EIMD might be similar to that of dairy protein. According to Shaw et al. (2016), 15 g of collagen is more effective than 5 g at stimulating collagen synthesis. However, conversely a single 30 g dose of collagen failed to increase rates of collagen

synthesis, nor did it improve recovery of DOMS after EIMD (Aussieker et al., 2023). Nonetheless, there are minimal guidelines for collagen in the context of EIMD (Bischof et al., 2024), which has made it challenging for some researchers to choose an appropriate dosage amount (Clifford et al., 2019).

2.3.3.4 Daily Dietary Protein Intake

Although it appears that factors such as protein type and the amount consumed per serve influence the effect of supplementation on recovery, the overall daily dietary intake of protein is believed to exert the most influence (Master & Macedo, 2020). Of studies that recorded their participants' daily dietary protein, intakes range from ~0.8 to 2 g· kg· day (Table 2.7 and 2.8). In regard to the daily dietary intake of protein and protein supplementation, two issues might arise. Firstly, if dietary protein intake is insufficient, supplemental protein will likely not be enough to improve recovery from EIMD (Saracino et al., 2020). This is a very probable explanation for the findings reported by Dahlstrom Burnley et al. (2010), who provided young untrained males with a small amount of supplemental protein (0.4 g· kg· hour) on top of their modest daily dietary protein intake (78 to 83 g), and as a result supplementation had no effect on recovery. Conversely, if an excessive amount of dietary protein is already consumed prior to supplementation, it leaves little to no room for additional protein to benefit recovery (Davies et al., 2020). This likely occurred in a study by Apweiler et al. (2018), where an intake of ~1.5 g· kg· day was already so high that 40 g of casein protein did not improve recovery after recreationally participants performed drop jumps. Likewise, 25 g of whey protein had no effect on recovery from EIMD in resistance-trained males that already consumed 1.9 g· kg· day of daily protein (West et al., 2017).

There have been recommendations made by the American College of Sports Medicine (ACSM) for the daily dietary intake of protein (Jäger et al., 2017), which researchers may follow to ensure the right amount is consumed so that protein supplementation may benefit recovery. Eddens et al. (2017) followed these recommendations, which allowed them to maintain a good level of dietary control during their study, despite having found no benefit from whey protein hydrolysate. However, while they might be useful, they are not always precise. For instance, Saracino et al. (2020) followed the recommendations of the ACSM for general population (0.8 g· kg· day) and supplemented 40 g of whey protein to total 1.10 g· kg· day of dietary protein.

However, even when intakes were slightly above recommendations, Saracino et al. (2020) assert it was not enough to improve recovery from EIMD in untrained middle-aged males.

2.2.3.5 Protein Timing

The timing of supplementation may also be a factor to consider when researchers apply dairy protein and/or collagen to expedite recovery from EIMD in an experimental setting. Both supplements have been consumed before or after exercise (Table 2.7 and 2.8). Although some studies assert that it is more beneficial to consume protein supplements soon after exercise (Cockburn et al., 2010). This assumption might be based on the previously accepted 'anabolic window,' theory, where protein must be consumed within one to two hours following exercise, when muscle cells are primed to take in amino acids for the synthesis of new myofibrillar proteins, for it to have any benefit on recovery (Aragon & Schoenfeld, 2013). However, the newer stance, similar to the amount per serve, is that the total daily dietary intake of protein is what determines the effect protein supplementation has on recovery, rather than the timing of ingestion (Schoenfeld & Aragon, 2018). Regardless, most researchers agree that the consumption of protein should be in close proximity to exercise, be that before or after, as a large delay between exercise and supplementation is unlikely to benefit recovery (Apweiler et al., 2019; Schoenfeld et al., 2017); which Saracino et al. (2022) demonstrated when evening protein ingestion after morning exercise failed to improve recovery from EIMD.

2.2.3.6 Duration of Supplementation

Alongside other dosage variables, the duration of supplementation may also influence the effects of dairy protein and/or collagen. Previous studies have used varying durations, ranging from one day to 12 weeks (Table 2.7 and 2.8). However, some researchers suggest that a longer duration of supplementation might be required to observe a benefit (Khatri et al., 2022). According to Gee et al. (2019), after finding no effect following an acute dose, dairy protein should be consumed over an extended period of time in order to improve recovery from EIMD. While prolonged ingestion may provide muscle cells with a more consistent supply of amino acids, compared to one-off doses that supply a limited number, research using longer durations is limited (Jäger et al., 2017) and acute supplementation has been shown to be beneficial in other

studies (Aussieker et al., 2023; Buckley et al., 2010; Etheridge et al., 2008). Nonetheless, a supplements' duration is an important variable researchers must consider when attempting to expedite recovery from EIMD.

2.2.3.7 Dietary Control

It is important that researchers use an appropriate level of dietary control when examining the effect of any nutritional intervention on recovery from EIMD. In doing so, this helps to minimise possible confounders, including insufficient/excessive daily dietary intakes of protein or diet inequality across groups, and facilitates the necessary conditions for an effect from supplementation to occur (Eddens et al., 2017). Brown et al. (2018) highlights the risk of inadequate dietary control, which prompted them to cater all meals during the study period, and as a result dairy protein supplementation had a beneficial effect on recovery from EIMD. Regardless of importance, not all studies have used such a high level of dietary control, if any at all (Table 2.7 and 2.8). For example, Nieman et al. (2020) chose not to record the diets of their participants at any stage of recovery and suggest that this could have consequently affected their study. Moreover, Apweiler et al. (2018) attempted to 'control' participants' diets with food diaries, but found it was not enough to stop a group difference in the daily intake of protein from occurring.

2.4 Limitations and Recommendations

While the literature outlined in the previous sections provides valuable insight into the effect of dairy protein and collagen on recovery from EIMD, and subsequent DOMS, it is necessary to acknowledge several limitations.

2.4.1 Dietary Control

Firstly, a frequently stated limitation of studies examining the effect of dairy protein and/or collagen on EIMD recovery is a lack of control around the daily dietary intake of protein (Apweiler et al., 2019; Cockburn et al., 2008; Dahlstrom Burnley et al., 2010; Gee et al., 2019; Hilkens et al., 2021; Nieman et al., 2020; Ormsbee et al., 2022; West et al., 2017). Among all possible

confounders, it has the greatest potential to interfere with the effect of supplementation (Eddens et al., 2017). This was demonstrated by Nieman et al. (2020), who used no form of control other than instructing participants to continue with their normal intake of protein during the 5-day study. While it is difficult to determine whether their participants consumed an excessive or insufficient amount of daily protein, whey protein supplementation (0.9 g· kg· day) consequently had no effect on recovery of DOMS or exercise performance after a 90-minute eccentric exercise protocol. Other studies have also encountered similar problems, where a lack of dietary control has had unfavourable outcomes following protein supplementation, prompting them to make several recommendations for future research (Apweiler et al., 2018; Ormsbee et al. (2022). Some studies have gone as far as to standardise diets and cater all meals to minimise this limitation, despite the expense (Brown et al., 2018; Saracino et al., 2020). Although, using such a high level of control does not always ensure that supplementation will be beneficial, particularly when the daily dietary intake of protein is already enough to facilitate recovery (Davies et al., 2020). Nonetheless, it is certain that simply instructing participants to continue with their normal daily intake of dietary protein is not the level of control that is required to minimise this limitation. Therefore, also based on previous recommendations, future dairy protein and/or collagen research would benefit from using diet records before and during the study period. Additionally, outside of adequate dietary control, it is also important that the amount of daily protein consumed leaves enough room for protein supplementation to have an effect.

2.4.2 Indirect Measures

After inducing EIMD, it is typical for researchers to assess it indirectly (McKune et al., 2012). While this approach may provide some valuable insight into symptoms that are not otherwise directly visible (e.g., DOMS), some of the measures used can be somewhat subjective and/or unreliable. Subjective measures of muscle soreness (e.g., VAS) are widely used to examine DOMS following EIMD, despite some of their inherent reliability issues (Cleather et al., 2007). A VAS is a basic measure of muscle soreness on a fixed scale of 0 to 10 (Clifford et al., 2019). Although it is simple to use, it does not capture all aspects of DOMS (Cleather et al., 2007). Therefore, it is possible that previous studies may have not been able to accurately assess DOMS when using a VAS, and this consequently impaired their ability to observe an effect following supplementation of collagen and/or dairy protein. One approach to improve this measure's

reliability would be to use multiple measures of muscle soreness to assess DOMS (Apweiler et al., 2019; Brown et al., 2018; Clifford et al., 2019); although different muscle soreness measures are not always correlated (VAS and PPT) and may produce different results (Clifford et al., 2019). Alternatively, a repeated measures study design (where the measure is repeated) could be used to reduce rating variability and improve accuracy. Nonetheless, some difficultly remains in the ability to accurately assess something (DOMS) that is naturally subjective.

Alongside muscle soreness measures, researchers may monitor changes in biomarkers to indirectly assess EIMD. This allows them to assess muscle damage and/or inflammation without having to perform more invasive or technical procedures, including muscle biopsies (Haller et al., 2023). Moreover, when biomarker analysis is used concurrently with other indirect measures, it increases researchers' ability to accurately quantify EIMD (Chalchat et al., 2022). However, biomarkers reflect systemic changes, as opposed to what might be occurring at a cellular level (Baird et al., 2012). They are also non-specific to EIMD and may indicate other non-related changes (e.g., acute illness or exercise itself; Haller et al., 2023). More importantly, many commonly used biomarkers (e.g., CK) display large inter-individual variability, where further data transformation is often required to remove this variability (Baird et al., 2012; Bischof et al., 2024; White et al., 2008). Indeed, existing studies have viewed their reliance on biomarkers to be a limitation of their research (Cooke et al., 2010; Ormsbee et al., 2022; Prowting et al., 2021; White et al., 2008). Some studies have also used it as an explanation for protein supplementation's lack of effect on EIMD recovery (Buckley et al., 2010). Consequently, many have recommended that future research use muscle biopsies, not only to account for variable biomarker responses, but to also determine whether supplementation has had a localised effect (Clifford et al., 2019; Prowting et al., 2021). While biopsies may address the problems of biomarkers, they come with their own limitations (e.g., technical or costly), which has restricted some studies (Prowting et al., 2021).

2.4.3 Training History

When examining EIMD in a controlled environment, it is important for researchers to consider the training history of the population that they have sampled from. Well-trained participants that are familiar to exercise do not damage as easily as novices that do not regularly participate in exercise (Nieman et al., 2020). Therefore, the chosen protocol used to induce EIMD should be appropriately matched to the participants' training history. For example, researchers

should use a higher intensity protocol when using experienced/well-trained participants or it may not be strenuous enough to cause EIMD (Ormsbee et al., 2022; ten Haaf et al., 2021). This was a likely scenario in a study by Davies et al. (2020), who admit that their resistance-trained participants were perhaps too familiar with the 7-day resistance exercise protocol used to induce EIMD. Furthermore, participants recruited by Betts et al. (2009) had a high level of training, which could have explained why CRP did not significantly increase above baseline after 90 minutes of shuttle running. Nonetheless, well-trained participants performing familiar exercise is a common occurrence in the existing literature, regardless of whether it is harder to induce EIMD or not (Bett et al., 2009; Davies et al., 2020; Eddens et al., 2007; Gee et al., 2019; Ormsbee et al., 2022; West et al., 2017).

It is also important for researchers to ensure that their participants' training history/experience is equal and that there is no variation in baseline muscular function, otherwise participants that are more familiar with the protocol may sustain less EIMD than those that are not (Hilkens et al., 2021). This occurred in a study by Betts et al. (2009), who recruited both highly trained cyclists unfamiliar with shuttle sprints and highly trained team sports players familiar with shuttle sprints, and consequently the extent of EIMD was not comparable between groups. Moreover, a baseline variation between groups in absolute squat strength contributed to variable reductions in 1RM performance after resistance exercise was used to induce EIMD (Ormsbee et al., 2022).

One way to remove these training history limitations is to only recruit untrained participants unfamiliar to most, if not all EIMD protocols; an approach Nieman et al. (2020) chose to adopt. Cooke et al. (2010) also chose to recruit untrained participants, which enabled them to observe significant EIMD following resistance exercise. Alternatively, pre-study testing could be used as a requirement for study inclusion, or more emphasis could be placed on the choice of EIMD protocol (ensuring that it is very strenuous), particularly when researchers sample from athletic populations.

2.4.4 EIMD Protocol

Whatever the choice of EIMD protocol, whether it involves resistance, plyometric, or endurance exercise, it must be strenuous enough to induce muscle damage in the population sample, so that there is a need for nutritional intervention. This is a relatively straightforward task,

if proven modalities of exercise that cause EIMD are factored in to the choice of protocol. Some choices that have shown to be effective (because they focus on muscle lengthening) are drop jumps, downhill running, and other eccentric exercise protocols (Apweiler et al., 2019; Bischof et al., 2024; Brown et al., 2018; Clifford et al., 2019; Eddens et al., 2017; Etheridge et al., 2008; Hilkens et al., 2021; Prowting et al., 2021). Resistance and endurance exercise protocols have also been used to induce EIMD (Aussieker et al., 2023; Cooke et al., 2010; Davies et al., 2020; Gee et al., 2019; Hirose et al., 2013; Lopez et al., 2015; ten Haaf et al., 2021; West et al., 2017), but with varying levels of success. Ormsbee et al. (2022) utilised a resistance exercise protocol, involving eight sets of eight barbell squats and chest presses, that they suggest was 'high in external validity.' Although they also admit that their modest approach, where they were careful not to disrupt the normal training of well-trained participants (professional rugby players and Crossfitters), may have caused them not to induce EIMD. Additionally, other resistance exercise protocols used to induce EIMD have also failed to evoke an inflammatory response (Buckley et al., 2010; Robberechts et al., 2023; Saracino et al., 2020). Nonetheless, regardless of the samples' normal training schedules or the modality of exercise that is used, it is imperative that researchers select a protocol that successfully induces EIMD for there to be a need for nutritional intervention.

2.4.5 The Repeated-Bout Effect

Following on from training history and protocol choice, the repeated-bout effect is another limitation that can undermine the effects of protein supplementation. It is a protective mechanism that lessens the subsequent physiological response when an exercise stimulus is repeated (Contrò et al., 2016). While the repeated-bout effect is an important adaptative response in a real-world context, experimentally it can reduce the validity of crossover studies (where a participant acts as their own control; Nieman et al., 2020), unless a single limb damage model is used; although this does not account for a reduction in systemic markers or neural adaptations during subsequent bouts of EIMD (Howatson & van Someren, 2007). Despite the negative influence of the repeated-bout effect, previous studies have still opted to use crossover designs (Dahlstrom Burnley et al., 2010; Draganidis et al., 2017; Etheridge et al., 2008; Hirose et al., 2013; West et al., 2017).
Not only can the repeated-bout effect cause less muscle damage in subsequent trials, but it can also make it appear that supplementation has improved recovery (when it has not) if the subsequent trial is also the intervention group. This may have occurred in crossover studies by

Draganidis et al. (2017) and Hirose et al (2013), although they both assert that a 'wash-out' period was enough to remove/reduce the repeated-bout effect. However, it may persist much longer than most wash-out periods, where it has been shown to last nine months after an initial bout of exercise (Nosaka et al., 2001). One approach that would completely remove the repeated-bout effect is an independent group design, requiring participants to perform exercise only once, which was precisely what Hilkens et al. (2021) chose to do. However, one downside to this approach is that it requires a larger number of participants per group, or sample sizes will be too small.

2.4.6 Sample Size

A study that has limited statistical power, due to small sample sizes, will find it challenging to observe a benefit of supplementation, most notably if data from indirect measures are extremely variable (Prowting et al., 2021). A number of existing studies have indicated that small sample sizes were a limitation of their research (Prowting et al.,2021; Saracino et al., 2020; ten Haaf et al., 2021). For example, ten Haaf et al. (2021) did not achieve their forecasted sample size, which led to a reduction in power. Fortunately, however, they were still able to observe a difference between groups. On the other hand, small sample sizes did limit the ability of Prowting et al. (2021) to draw statistical conclusions from their data. Nonetheless, unforeseen circumstances may make it impossible for studies to maintain large enough sample sizes. This was demonstrated in Saracino et al. (2020), where several participants had to withdraw following safety concerns, which reduced statistical power for their primary measure of EIMD. A crossover study design could be used to increase power and minimise this limitation, but it is often discouraged due to the repeated-bout effect (Prowting et al., 2021). Another way would be to recruit a larger number of participants to account for any dropouts.

2.4.7 PLA or CON Type

A PLA-controlled group serves multiple purposes in research. It is useful for removing an expectation of a benefit and can be used to test the effect(s) of an intervention against normal recovery (Gerdesmeyer et al., 2017). Therefore, in order for a PLA to be effective/suitable, it must be able to imitate the intervention (e.g., same taste, smell, volume, and appearance). It should also be isoenergetic so that the extra energy from the intervention is not the reason why recovery

improves (Gee et al., 2019). However, the effects of dairy protein and/or collagen have often been compared to an ineffective/unsuitable PLA or CON. For example, the effects of milk protein on recovery from EIMD were compared against water, and as a result the intervention provided participants' with considerably more energy (Cockburn et al., 2010). Despite the use of ineffective/unsuitable PLAs or CONs, it is a limitation that has been widely acknowledged in the existing literature (Cockburn et al., 2008; Cockburn et al., 2010; Hirose et al., 2013; Robberechts et al., 2023). Therefore, to address this limitation, future studies should consider using an appropriate isoenergetic PLA-controlled group.

2.4.8 Study Duration

The existing literature has used study periods of varying durations. Although the length of time needed to recover from EIMD is not fixed, most researchers have observed it to be longer than 24 hours (Eddens et al., 2017; Etheridge et al., 2008; White et al., 2008). Therefore, the duration of a study should be long enough to capture the full time course of recovery without makers of EIMD (particularly biomarkers) continuing to rise after the final measures have been taken. Otherwise, an opportunity to observe a benefit of a supplement could be missed. This was likely the case in a study by Buckley et al. (2010), who may have missed the chance to observe both a change over time (due to EIMD) and an attenuation (due to supplementation) in CK, as it was only 24 hours long.

2.5 Summary of the Literature

EIMD is an acute muscular injury that is a negative consequence of strenuous, repetitive, novel, and/or eccentric exercise (Tanabe et al., 2022). Whist irritating to say the least, it has the potential to reduce subsequent exercise performance and cause a disruption to the normal daily activities of regular exercisers (Hody et al., 2019; Tesarz et al., 2012). The assessment of DOMS and muscular function, used in conjunction with biomarker analysis, enables researchers to quantify EIMD; despite some variable timelines of presentation and recovery (Allen et al., 2004). EIMD likely arises from ultrastructural muscle damage that triggers inflammation, but this is not a neat two-phase process. Furthermore, although it can be induced in a controlled environment for the purpose of improving a researchers' understanding of its mechanisms, there is no consensus

on how it should be induced, let alone how it should be measured. Nevertheless, researchers have agreed upon putting forth their utmost effort to find an effective nutritional intervention to expedite recovery from EIMD, and subsequent DOMS. A lot of their focus has been on dairy protein. Marketed as a post-exercise supplement that promotes muscle repair, it may enhance muscle protein synthesis, which is believed to improve recovery from EIMD. However, most of the findings on dairy protein have been somewhat inconsistent, and it has been shown to have a modest effect on DOMS (Brown et al., 2018). Alternatively, collagen, a by-product created by the meat industry that is believed to improve connective tissue repair by enhancing collagen synthesis, has been shown to benefit recovery of all markers of EIMD (Lopez et al., 2015), most notably DOMS (Clifford et al., 2019; Lopez et al., 2015). Therefore, if collagen is at least equivalent, if not more effective than dairy protein, it will have important practical implications for experts in the field of sport and exercise, including exercise practitioners that make recommendations to regular exercisers recovering from EIMD, and subsequent DOMS. Consequently, the overall impact of collagens' benefits may, in turn, improve the utilisation of waste by-products created by the meat industry. However, based on the small number of existing studies on collagen in the context of EIMD, let alone the even fewer number comparing it directly to dairy protein, it is difficult to find evidence to support its equivalency, nor superiority over dairy protein.

3. Research Aim and Hypotheses

The aim of the study was to compare the effect of collagen hydrolysate (CH) to milk protein concentrate (PRO) on acute recovery from EIMD, and subsequent DOMS, induced by downhill running in recreationally active males. It was hypothesised that 25 g of protein from CH would reduce ratings of DOMS, increase recovery for measures of muscular function, and attenuate biomarkers of muscle damage and inflammation following EIMD, to an equal or greater extent than an equivalent amount of protein from PRO, and that all protein sources would be more effective than a placebo (PLA).

4. Methods

4.1 Study Overview

A double-blinded, PLA-controlled, independent group design was used to compare the effect of CH to PRO on acute recovery from EIMD, and subsequent DOMS, after thirty-three recreationally active males completed a 30-minute-downhill run. Participants were randomised into one of three groups (PRO, PLA, CH), and attended a total of five visits. The first of these visits was a familiarisation session, where baseline characteristics were collected, and participants were familiarised with the exercise measures and EIMD protocol before they went on to perform a VO_2max test. The second visit began with pre-exercise criterion measures (bloods, VAS, CMJ, IMTP, MVIC, and running economy) that were followed by a downhill run at 80% of pre-determined VO_2max speed on a -15% slope for 30 minutes. Criterion measures were then repeated before participants consumed their first allocated supplement (CH, PRO, PLA). Two hours before returning to the laboratory to complete follow-up measures (at 24, 48, and 72 hours post-downhill run), participants consumed their supplement.

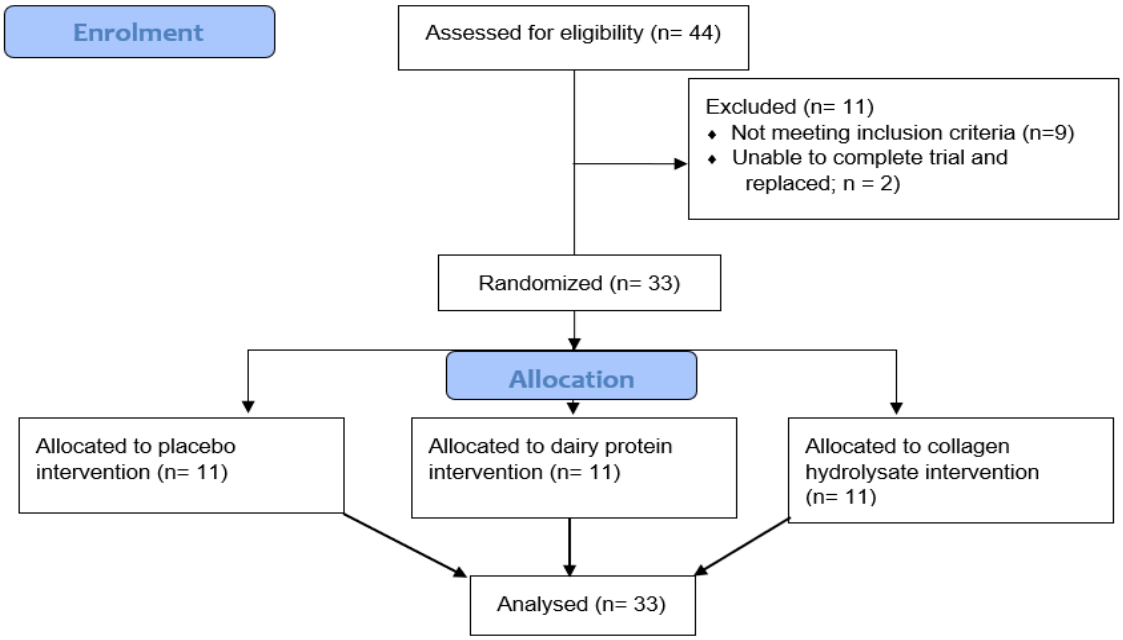
4.2 Participants

Thirty-three recreationally active males, aged between 18-40 years, free of illness or injury that could be exacerbated by high intensity exercise participated in the study. Those who regularly consumed protein or other ergogenic supplements (e.g., pre-workout products, branched chain amino acids, or creatine monohydrate), consumed more than 1.6 g· kg· day of dietary protein, who were vegan or vegetarian, and who were participating in other research studies were all excluded from the study. All participants were volunteers recruited by means of public advertisement (Appendix A), who received an information booklet (Appendix B) after expressing their interest in the study. Upon their agreement to participate, both a completed health screening form (Appendix C) and written informed consent (Appendix B) were obtained. Ethical approval was granted from the New Zealand Health and Disability Ethics Committee (EXP 12330) and registered with the Australian and New Zealand Clinical Trials Register (ACTRN12622000529741). See Figure

4.1 for recruitment details. Following their recruitment, participants were then randomly assigned to one of three groups (PRO, PLA, CH) that were counterbalanced to ensure an even distribution per group.

Figure 4.1

CONSORT Flow Diagram



Note. CONSORT flow diagram displaying the movement of participants through the study.

4.3 Experimental Design

All participants attended a familiarisation session (Visit 1) in the Human Performance Laboratory, School of Sport, Exercise and Nutrition at Massey University, Palmerston North. At the beginning of the session, baseline characteristics (Table 5.1) were collected followed by a familiarisation of each measure and the EIMD protocol. At the end of the session, participants completed a VO_2max test to determine their future running speeds for the running economy test and downhill run. Within one week after familiarisation, participants returned to the laboratory for the main trial (Visit 2), morning or afternoon, at a time that was convenient to them. Prior to

the downhill run, pre-exercise measures (blood sampling, VAS, CMJ, midthigh pull, MVIC, and running economy) were performed. Participants then completed 30 minutes of downhill running at a speed equivalent to 80% of their predetermined VO_2max on a -15% slope. Following a 30-minute rest, pre-exercise measures were repeated. Participants then consumed their first allocated supplemental drink (PRO, PLA, CH). Two hours prior to returning to the laboratory for each of the three follow ups (Visits 3, 4, and 5), participants were instructed to consume their allocated supplement drink. See Table 4.1 for study timeline.

Table 4.1

Study Timeline

Study Variable	Visit 1		Visit 2		Visit 3*	Visit 4*	Visit 5*
	Familiarisation	PRE	EIMD	30 min	24 hr	48 hr	72 hr
VO_2max	x						
Supplement				x	x	x	x
Measures		x		x	x	x	x

Note. *Supplement was consumed two hours prior to each of these visits. Pre-exercise (PRE), exercise-induced muscle damage (EIMD).

4.3.1 Supplements

The supplement powders: PRO (milk protein concentrate 470; Fonterra Cooperative Group Ltd, New Zealand), PLA (maltodextrin), and CH (bovine collagen hydrolysate; FoodPilot Massey University, New Zealand) were sealed in foil packaging labelled A, B, or C to ensure double blinding. Prototype spray-dried enzymatic hydrolysate of collagen was prepared conventionally from frozen, fresh dehaired cow hide in the FoodPilot, Massey University, Palmerston North, New Zealand. Hide was provided by Southern Pastures (NZ) Ltd (New Zealand Business Number 9429031978873). All supplements were isoenergetic and flavour-matched with artificial and natural vanilla flavourings (Table 4.2 for nutritional information). Both protein supplements (PRO, CH) were protein matched (25 g), and all supplements were isoenergetic. Each serving was mixed with 250 ml of water in a provided shaker and then consumed.

Table 4.2

Nutritional Information of Supplements

Component	PRO	PLA	CH
Serving (g)	35.8	53.2	26.9
Energy (kJ)	1000	1000	1000
Protein (g)	25.0	0.0	25.0
CHO (g)	32.9	58.8	33.8
Fat (g)	0.42	0.0	0.0

Note. Milk protein (PRO), placebo (PLA), collagen hydrolysate (CH), carbohydrate (CHO).

Table 4.3

Amino Acid Content of Protein Supplements

Amino Acid (mg/100 mg)	PRO	CH
Aspartic Acid	78	5.95
Threonine	45	1.74
Serine	56	3.03
Glutamic Acid	216	8.12
Proline	98	12.75
Glycine	19	22.68
Alanine	33	8.57
Tryptophan	14	-
Valine	64	2.72
Methionine	28	0.91
Isoleucine	53	1.51
Leucine	96	2.95
Tyrosine	53	1.23
Phenylalanine	49	2.14
Histidine	27	0.68
Lysine	84	4.08
Arginine	37	8.18
Hydroxyproline	-	10.79
Cysteine	12	0.02

Note. Milk protein (PRO), collagen hydrolysate (CH).

4.3.2 Dietary and Exercise Control

Twenty four-hour diet records (Appendix D) were used to ascertain participants' normal daily dietary protein consumption (g/day), energy intake (kJ/day), and whether ergogenic supplements were used. All diet records were analysed by a nutritional expert and used to determine study inclusion. During the study period, participants were instructed to maintain their habitual diets. To ensure an equal intake of energy prior to the downhill run and each subsequent visit, at the end of the familiarisation session, participants were given a pre-run drink that was energy-matched to the supplements. This was provided in a shaker and contained 61.6 g (1000 kJ) of maltodextrin powder, which they were told to mix with 250 ml of water and consume two hours before Visit 2. Participants were also instructed to refrain from strenuous exercise, alcohol, and anti-inflammatory drugs 48 hours before Visit 2 and until after Visit 5.

4.3.3 VO_2max

A graded treadmill running test was used to determine participants' VO_2 at four submaximal running speeds and VO_2max (L/min). Results were used to calculate the running speeds of the downhill run and running economy test. During the test, participants were fitted with a silicone mask that covered their nose and mouth and a heart rate monitor around their chest. The mask was connected to a metabolic gas exchange analyser (Quark, Cardio Pulmonary Exercise Testing by Cosmed, Italy) that continuously collected and analysed inspired and expired air. Heart rate was also continuously recorded during the test. Participants ran on a standard motorised treadmill at 1% incline (to mirror normal outdoor running) for four increasingly faster, predetermined, submaximal speeds, that lasted four minutes per stage. After the last submaximal stage, the speed was then increased by one km/hour every minute until they reached volitional fatigue. The relationship between submaximal oxygen consumption and running speed were used to formulate a linear regression equation. This equation, in conjunction with the VO_2max, was then used to calculate the speed at 60% and 80% VO_2max for each participant.

4.3.4 EIMD Protocol

Muscle damage was induced with an adapted downhill running protocol that has been successfully used and validated by similar studies (Chen et al. 2007; Chrismas et al. 2017; de Lima

et al., 2021; Lima et al., 2019). Downhill running was chosen to induce EIMD because it allows for participants of varying backgrounds to exercise at a speed that is relative to their fitness level without maximal effort. Participants ran at 80% of their predetermined VO_2max speed on a motorised treadmill (VacuMed, California, USA) on a -15% slope for 30 minutes. The slope and time was chosen because it induces muscle damage in a shorter period of time, compared to longer downhill running protocols (Southall-Edwards et al., 2022). However, the percentage of VO_2max speed was adapted from similar studies by increasing the speed by 10% to ensure EIMD occurred.

4.3.5 Muscle Soreness

A 100mm VAS was used to measure self-reported muscle soreness (Appendix E). After preforming a squat hold of three seconds at a 90° knee angle, participants were asked to rate muscle soreness of their lower body muscles on a scale of 0 to 10 (0: no soreness, 10: extreme soreness) by drawing a vertical line across a 100 mm horizontal line (Hilkens et al., 2021). Participants also rated muscle soreness of their right quadriceps, after preforming three MVICs of the right knee extensors (Cockburn et al., 2010).

4.3.6 Muscular Function

4.3.6.1 CMJ

A CMJ was used to measure lower body power output. The jump was performed on a digital jump mat (SmartSpeed, Australia) that recorded jump height in cm. Participants stood on the mat with their hands on their hips. Using a squat of self-selected depth, they then performed a maximal vertical jump without tucking their knees while in the air. The CMJ was repeated three times, with 30 seconds rest in between jumps. The highest jump was then used for analysis. A CMJ is a valid measure of lower body power, previously used by Young et al. (1995) and replicated by Prowting et al. (2021).

4.3.6.2 IMTP

An IMPT was used to measure peak force of the lower body, utilising a similar protocol to that established by Haff et al. (1997). A custom-made platform and load cell was used, as it has been shown to be an equally valid and reliable measure of force, comparable to expensive force plates (Couto et al., 2023). To begin the IMTP, participants stood on the small platform with their hips and knees bent at joint angles recommended by Beckham et al. (2013; hips: 120°, knees: 140°). This enabled the bar gripped by the participant to sit approximately at midthigh level. A goniometer was used to ensure joint angles were consistent throughout each trial. The bar was connected to the platform at one end and a load cell at the other by a chain (Couto et al., 2023). After a three second countdown, participants were instructed to produce maximal force by pulling the handle upwards for three seconds. Force was displayed on a digital monitor. Following 30 seconds of rest, the IMPT was repeated until three attempts were made. The highest peak force (N) was used for analysis.

4.3.6.3 MVIC

MVIC of the right knee extensors was assessed to measure isometric force, with a custom-made isometric dynamometer as used by Barnes et al. (2019). Participants were secured by a seat belt in an upright position so that the angles of the knees and hips were at 90°. A strap was also placed around their right ankle, attaching them to a lever arm, connected to a load cell that was connected to a custom-made amplifier. Data was recorded using Powerlab data collection unit (ADInstruments, Australia) and force was recorded in Chart for Windows (v8, ADInstruments, Australia). After receiving a three second countdown, participants maximally extended their right knee for three seconds. They repeated this three times, with a 30 second rest in between attempts. The highest peak force (N) of all three attempts was used for analysis.

4.3.6.4 Running Economy

Running economy, a test of cardiovascular function, was measured by collecting and analysing inspired and expired air using the same gas analyser used in the VO_2max test. As with the VO_2max test, participants also wore a face mask and heart rate monitor. Participants ran on a

motorised treadmill set at 1% incline to reflect outdoor running (Marcora & Bosio, 2007) at 60% of their previously determined VO_2max speed for five minutes to warm-up. After which, the speed was increased to 80% of VO_2max speed for an additional five minutes. This intensity was chosen because it has been shown to be impacted following downhill running in previous research (Chen et al., 2009; de Lima et al., 2021). VO_2 (L/min) was averaged over the last minute and then used for analysis.

4.3.7 Biomarker Analysis

Blood samples were drawn at all five timepoints of recovery (PRE, 30 minutes, 24, 48, 72 hours) from an antecubital vein into vacutainer tubes (24 ml total) by a trained phlebotomist. They were centrifuged and stored at -80° until analysis. They were analysed for the presence of muscle damage (CK) and inflammatory (hsCRP) biomarkers by Canterbury Health Laboratories. Plasma CK was determined by enzymatic spectrophotometry on a Beckman Coulter AU5822 using Beckman Coulter reagents. hsCRP concentration was measured with the immunoturbidimetric method on Beckman Coulter AU5822 analyser using Beckman Coulter reagents.

4.4 Statistical Analysis

Participant number was calculated using G*Power software, version 3.0.10 for repeated measures design. Using 80% power, moderate effect size, and an alpha of 5%, a total of n = 9 participants per group were needed to determine significant differences in responses between groups. However, to account for participant drop out and/or non-compliance, the study aimed to recruit 33 participants as a minimum and 36 participants as a maximum. Baseline participant characteristics were compared using one-way analysis of variance (ANOVA) to ensure homogeneity between groups.

Prior to data analysis data were examined for normality using the Shapiro-Wilk test. Data that were non-normally distributed (CK & hsCRP) were log transformed and analysed (Vasquez et al., 2021). Mauchley's test was also used to assess sphericity (ε) and, where the assumption of sphericity was violated, adjustments to the degrees of freedom were made ($\varepsilon > 0.75$ = Huynh-Feldt; $\varepsilon < 0.75$ = Greenhouse-Geisser). After examining for normality, a two-factor mixed ANOVA with repeated measures was used to identify any time (PRE, 30 minutes, 24, 48, 72 hours), group

(MP, PLA, CH) or time x group interaction effects. Where a main or an interaction effect was identified, a post-hoc analysis using the Bonferroni adjustment was carried out. Partial Eta Squared was used to determine the effect size (small effect: $\eta p^2 = > 0.01$, medium effect: $\eta p^2 = > 0.06$, large effect: > 0.14). Statistical significance was set to $P < 0.05$. All data are reported as mean, presented as raw, percentage change, or transformed where appropriate. SPSS software was used for statistical analysis.

5. Results

5.1 Participants

At baseline, there were no significant differences in participant characteristics among the three treatment groups (PRO, PLA, CH). Characteristics, VO_2 max, MVIC, protein consumption, and daily energy intake are displayed in Table 5.1.

Table 5.1

Participant Baseline Characteristics

Characteristics	PRO (*n* = 11)	PLA (*n* = 11)	CH (*n* = 11)	*P* value
Age (years)	26.2 (7.2)	23.5 (6.1)	26.6 (6.4)	0.468
Height (cm)	181.1 (11.4)	182.0 (7.6)	176.0 (5.3)	0.220
Weight (kg)	87.0 (12.6)	82.3 (13.1)	82.6 (9.0)	0.581
VO2 max (ml· kg· min)	45.8 (5.2)	46.3 (5.3)	45.2 (5.7)	0.896
MVIC (N)	605 (85.5)	613.3 (82.5)	570.7 (82.9)	0.750
Protein (g· day)*	117.9 (55.8)	103.7 (38.3)	102.8 (38.4)	0.697
Protein (g· day· kg· bw)*	1.4 (0.6)	1.3 (0.6)	1.3 (0.5)	0.905
Energy (kJ· day)*	10767.7 (5627.5)	8340.8 (2143.0)	9988.8 (2687.5)	0.353

Note. Milk protein (PRO), placebo (PLA), collagen hydrolysate (CH). * Calculated from 24-hour diet record one week prior to the study-period as part of inclusion criteria. Data presented as means (SD).

5.2 Muscle Soreness

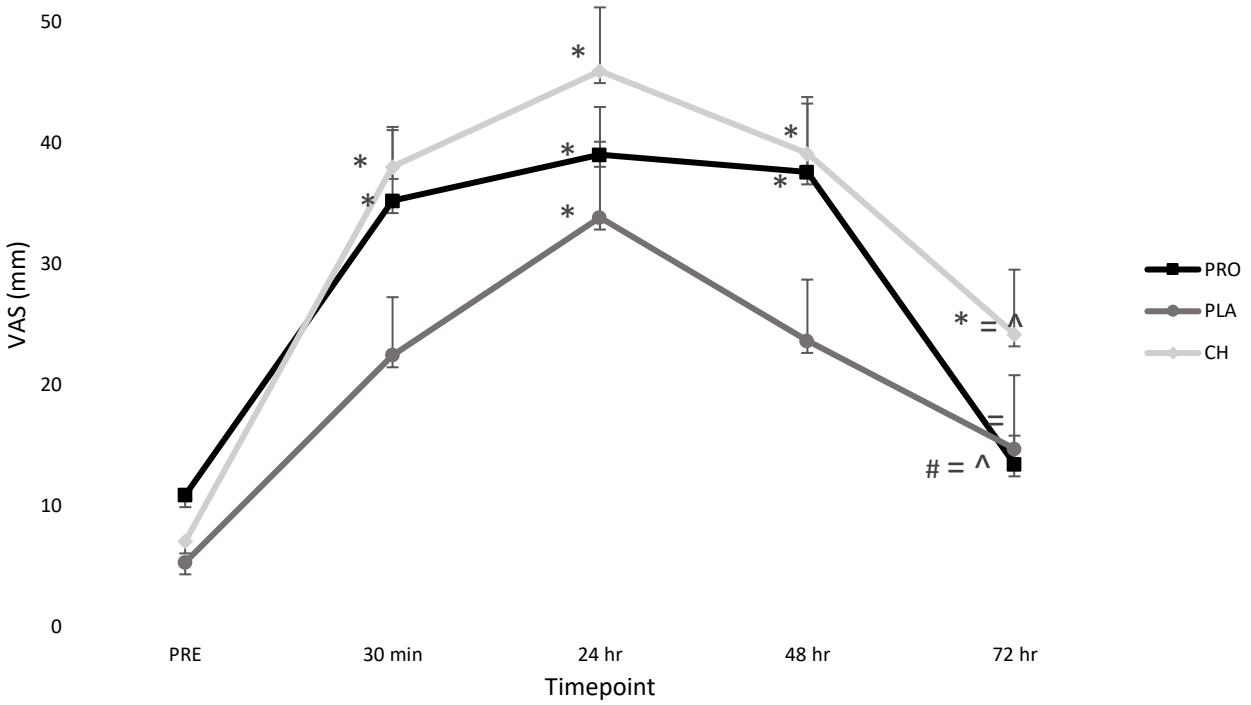
Muscle soreness significantly increased over time following the downhill run (Figure 5.1). Large significant time effects were found for muscle soreness during a squat (P = < 0.001, ηp^2 = 0.514) and MVIC (P = < 0.001, ηp^2 = 0.424). However, no significant group effects were observed for either measure of muscle soreness (squat: P = 0.063, ηp^2 = 0.168; MVIC: P = 0.065, ηp^2 = 0.189). Similarly, no interaction effects were found (squat: P = 0.401, ηp^2 = 0.066; MVIC: P = 0.225, ηp^2 = 0.94).

The highest rating of muscle soreness was observed during a squat at 24 hours (CH) and at 30 minutes post-run during MVIC. Muscle soreness during a squat did not return to pre-run ratings by the final time point of recovery in CH group (Figure 5.1), but it was not different to PRE at 72 hours in PRO and PLA groups. Muscle soreness during MVIC, however, recovered at 72 hours across all groups.

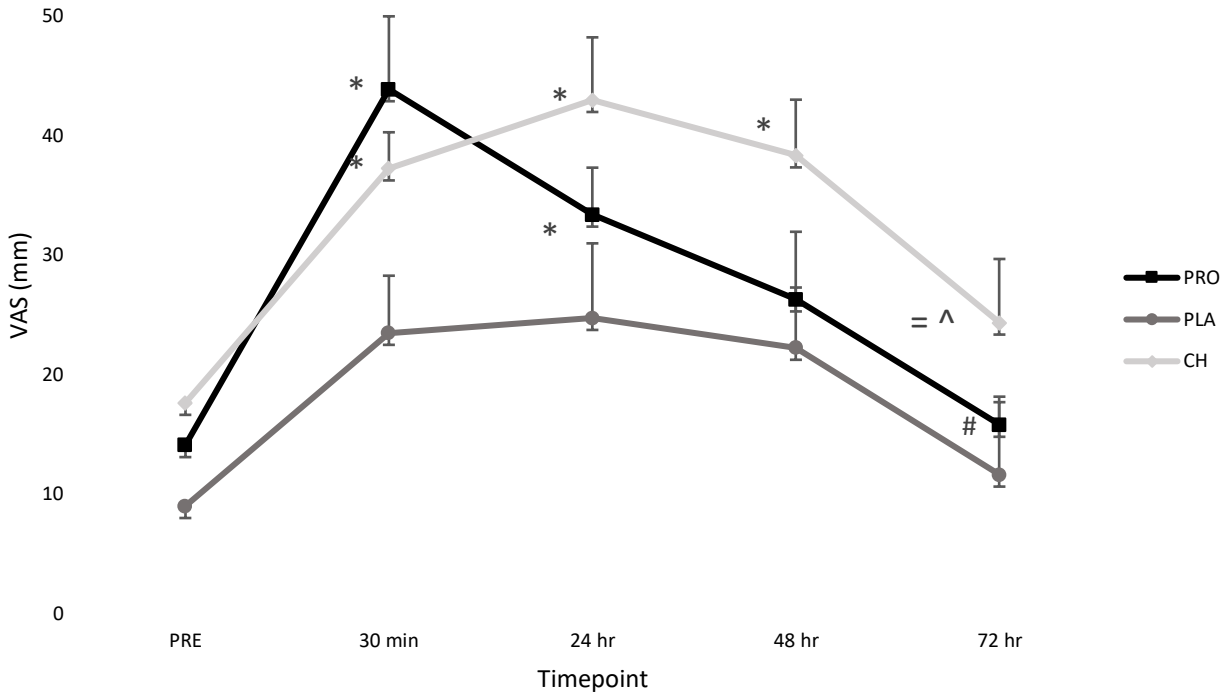
Figure 5.1

Perceived Muscle Soreness Following EIMD

a) *Squat*



b) *MVIC*



Note. Change in muscle soreness from pre-exercise (PRE) to post-exercise on a 0-100 mm visual analogue scale (VAS) for dairy milk protein (PRO), placebo (PLA), and collagen hydrolysate (CH) groups. Data presented as means (SD). * Significantly different (P = < 0.05) to PRE, # significantly different (P = < 0.05) to 30 min, = significantly different (P = < 0.05) to 24 hr, ^ significantly different (P = < 0.05) to 48 hr.

5.3 Muscular Function

All measures of muscular function significantly reduced over time following the downhill run (Table 5.2 & Figure 5.2). Large significant time effects occurred for raw CMJ (P = < 0.001, ηp^2 = 0.182), IMTP (P = < 0.001, ηp^2 = 0.180), MVIC (P = < 0.001, ηp^2 = 0.430), and running economy (P = < 0.001, ηp^2 = 0.229). Similar results were observed for percentage change in CMJ (P = < 0.001, ηp^2 = 0.166), IMPT (P = < 0.001, ηp^2 = 0.173) MVIC (P = < 0.001, ηp^2 = 0.394), and running economy (P = < 0.001, ηp^2 = 0.218). However, we did not observe any group effects for raw CMJ (P = 0.332, ηp^2 = 0.71), IMTP (P = 0.574, ηp^2 = 0.036), MVIC (P = 0.559, ηp^2 = 0.038), and running economy (P = 0.153, ηp^2 = 0.121), nor percentage change in CMJ (P = 0.658, ηp^2 = 0.028), IMPT (P = 0.913, ηp^2 = 0.006), MVIC (P = 0.741, ηp^2 = 0.020), and running economy (P = 0.382, ηp^2 = 0.064). Additionally,

no significant interaction effects were found for raw CMJ (P = 0.493, ηp^2 = 0.059), IMPT (P = 0.994, ηp^2 = 0.011), MVIC (P = 0.139, ηp^2 = 0.095), and running economy (P = 0.978, ηp^2 = 0.017), nor percentage change in CMJ (P = 0.500, ηp^2 = 0.058), IMTP (P = 0.997, ηp^2 = 0.009), MVIC (P = 0.123, ηp^2 = 0.020), and running economy (P = 0.957, ηp^2 = 0.022).

Most measures of muscular function had the greatest reduction 30 minutes post-run (Figure 5.2), with a -8% decrease in IMTP in the PLA group, -16% in MVIC in the PRO group, and running economy increased by 6% in the PRO group. However, the greatest decline in CMJ height (-6%) occurred at 48 hours in the PRO group. All measures of muscular function returned to PRE within 72 hours.

Table 5.2

Changes in Muscular Function Following EIMD

Measure	Timepoint	Group		
		PRO (n = 11)	PLA (n = 11)	CH (n = 11)
CMJ (cm)	PRE	35.29 (5.20)	38.12 (6.45)	37.49 (4.08)
	30 min	34.60 (5.98)*	36.37 (4.18)	36.42 (4.03)
	24 hr	33.16 (5.05)*	37.26 (5.49)	35.39 (4.59)*
	48 hr	33.16 (5.90)*#	36.50 (5.07)	35.25 (4.36)
	72 hr	33.70 (6.82)#^	37.50 (5.88)	36.63 (4.83)
IMTP (N)	PRE	1639.67 (535.98)	1535.72 (215.20)	1705.12 (450.20)
	30 min	1495.10 (396.23)	1415.73 (191.34)	1615.60 (494.63)
	24 hr	1504.51 (432.53)	1439.61 (292.10)	1611.32 (468.78)
	48 hr	1539.66 (478.27)	1431.59 (292.03)	1588.49 (385.01)
	72 hr	1600.97 (441.54)	1513.60 (288.80)	1698.16 (498.68)
MVIC (N)	PRE	605.00 (85.53)	613.29 (82.50)	570.68 (82.87)
	30 min	507.52 (83.30)*	555.08 (66.62)*	483.93 (101.28)*
	24 hr	559.02 (100.93)#	548.06 (78.70)*	526.87 (105.78)
	48 hr	580.69 (93.13)#	561.81 (102.50)	539.19 (111.38)#
	72 hr	604.40 (81.88)#	586.13 (110.56)	566.57 (95.71)#
Running economy (L/min)	PRE	3.20 (0.54)	2.98 (0.38)	2.90 (0.32)
	30 min	3.38 (0.52)*	3.09 (0.40)	3.07 (0.38)*
	24 hr	3.29 (0.50)	3.00 (0.34)	2.94 (0.36)
	48 hr	3.29 (0.48)	3.01 (0.37)	2.95 (0.35)
	72 hr	3.28 (0.57)	2.99 (0.35)	2.95 (0.45)

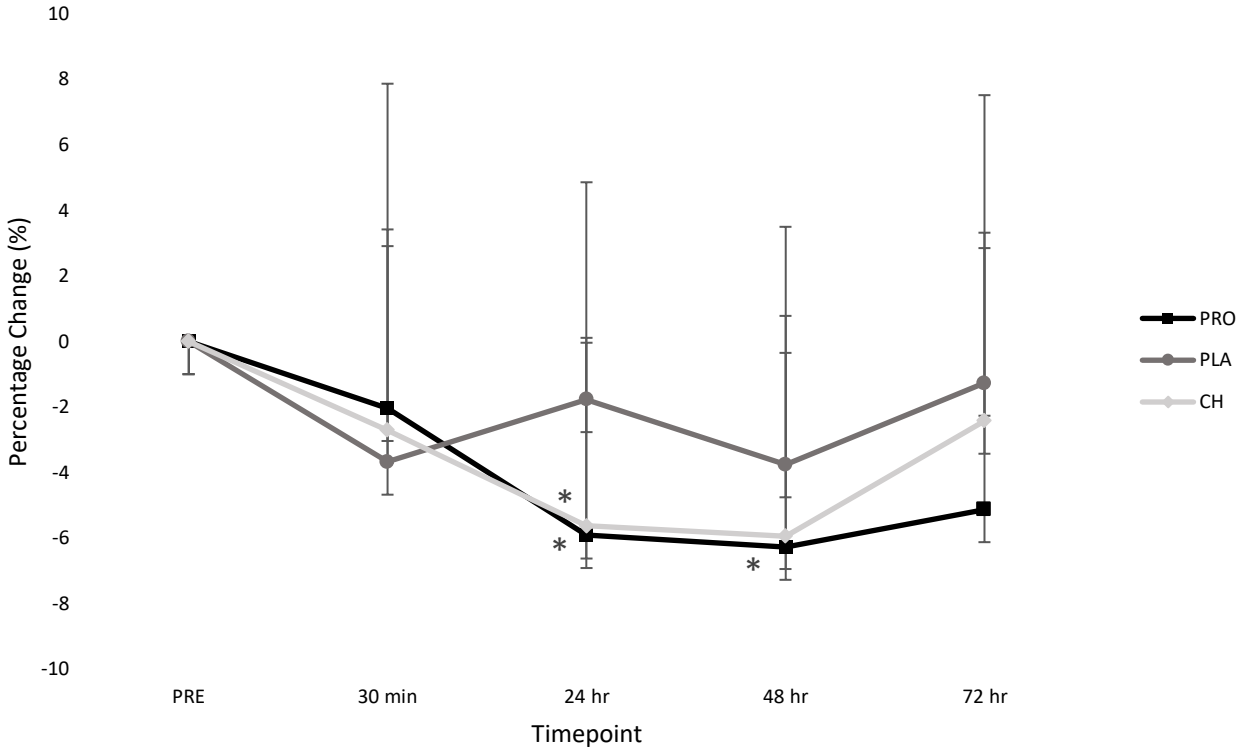
Note. Milk protein (PRO), placebo (PLA), collagen hydrolysate (CH), countermovement jump (CMJ), pre-exercise (PRE), isometric midthigh pull (IMTP), maximal voluntary isometric contraction

(MVIC). Data are presented as means (SD). * Significantly different (*P* = < 0.05) to PRE, # significantly different to 30 min, = to significantly different to 24 hr, ^ significantly different to 48 hr.

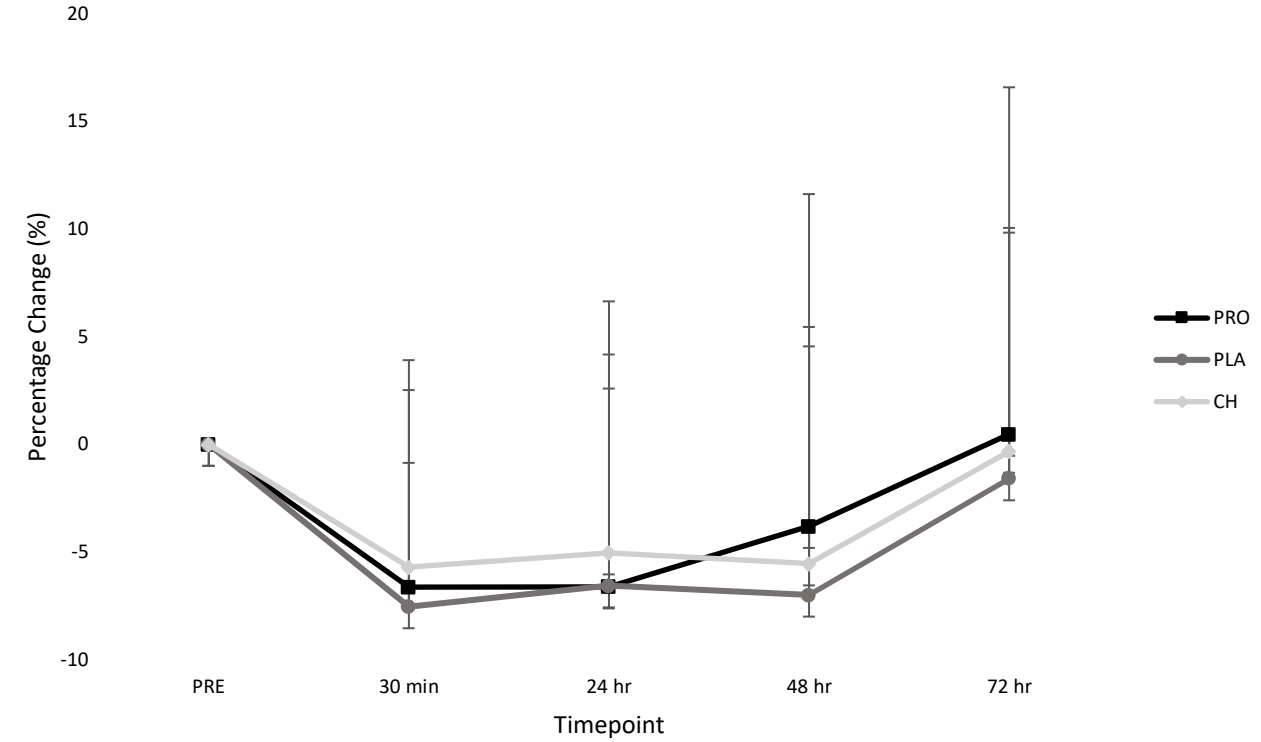
Figure 5.2

Percentage Change in Muscular Function Following EIMD

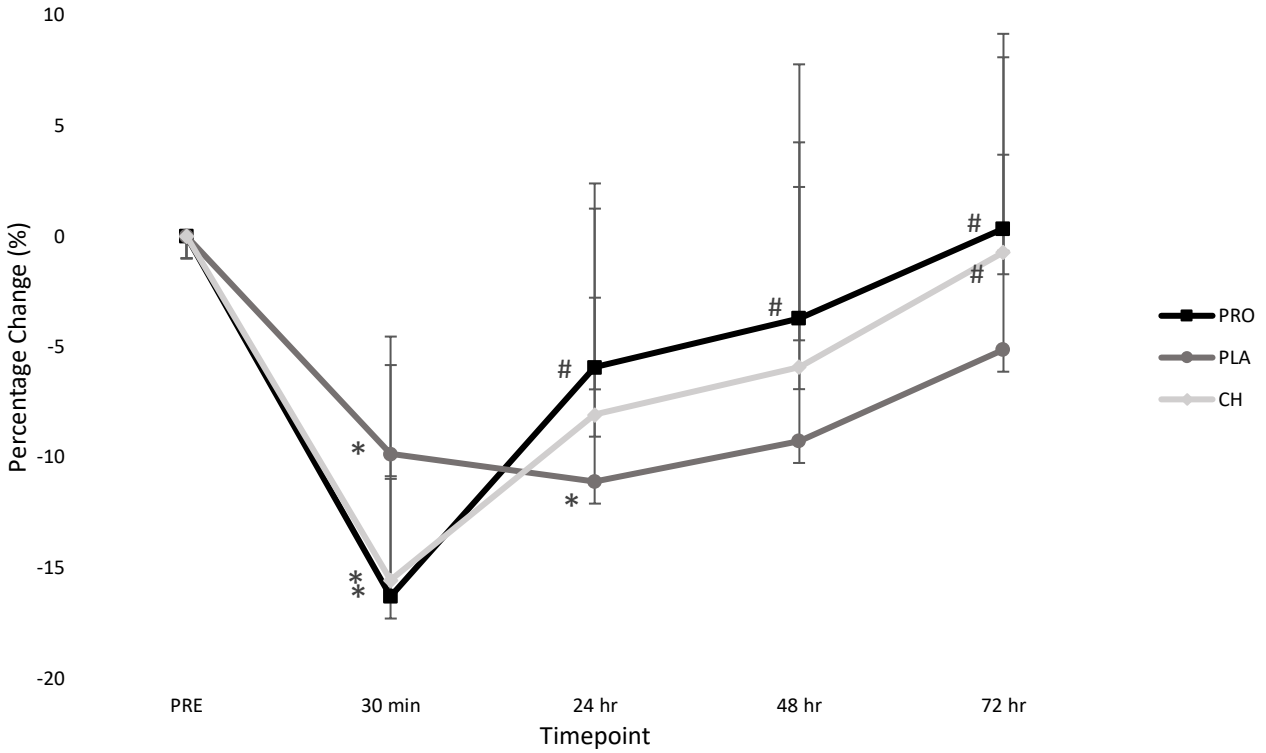
a) *CMJ*



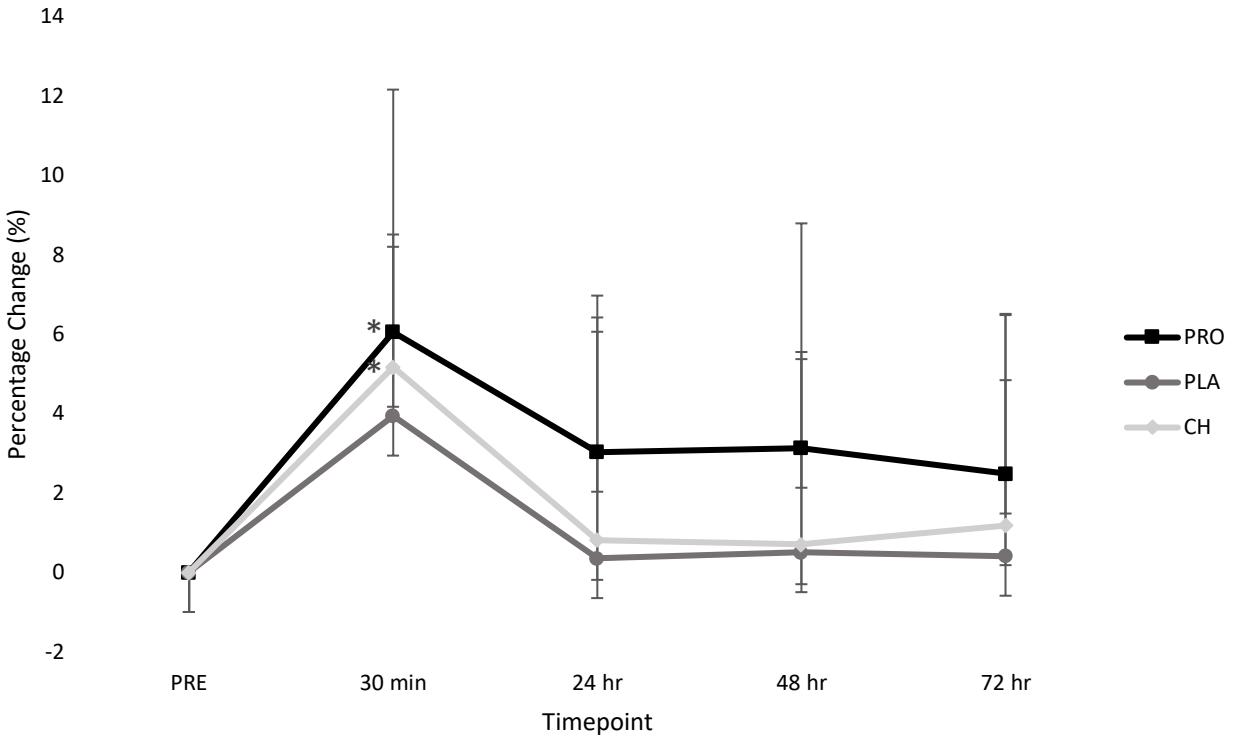
b) *IMPT*


20
15
10
5
0
-5
-10
Percentage Change (%)
PRE
30 min
24 hr
48 hr
72 hr
Timepoint
PRO
PLA
CH

c) *MVIC*


10
5
0
-5
-10
-15
-20
Percentage Change (%)
*
**
*
#
#
#
PRE
30 min
24 hr
48 hr
72 hr
Timepoint
PRO
PLA
CH

d) *Running Economy*



Note. Muscular function (%) change from pre-exercise (PRE) to post-exercise for milk protein (PRO), placebo (PLA), and collagen hydrolysate (CH) groups. PRE performance assumed as 0. Data are presented as means (SD). * Significantly different (P = < 0.05) to PRE, # significantly different to 30 min.

5.4 Biomarkers

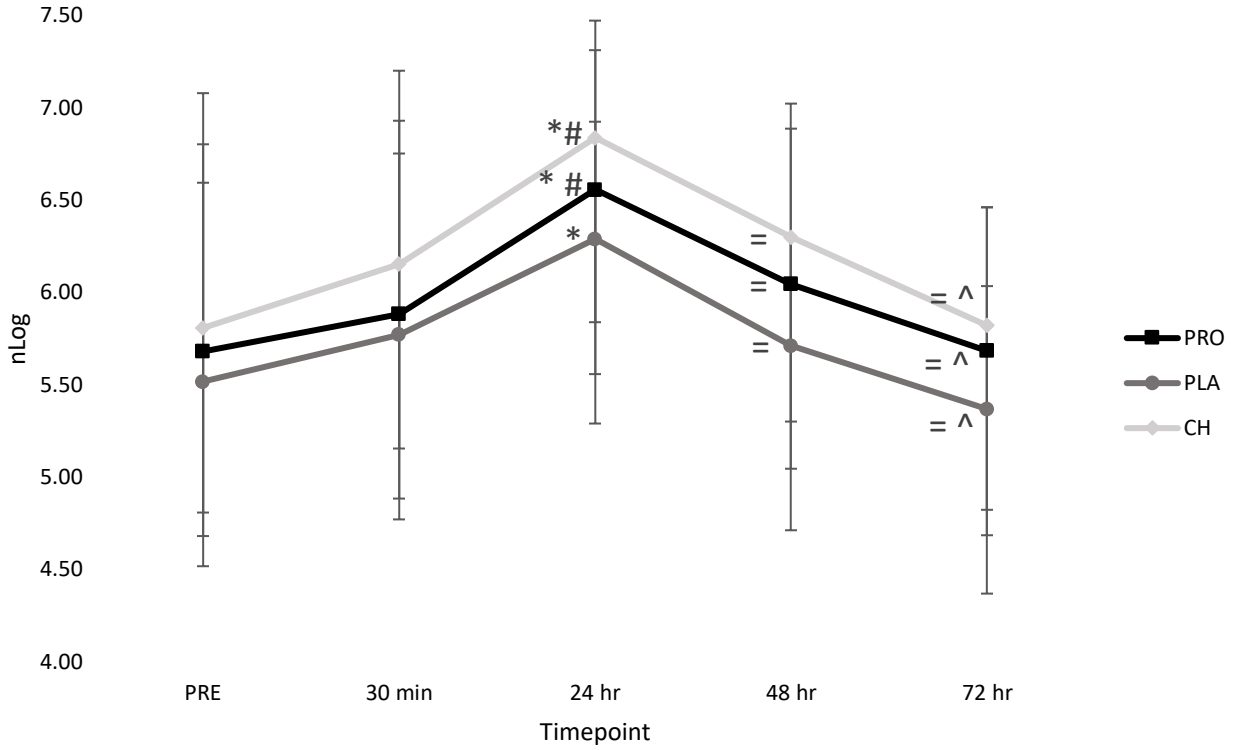
Blood analysis identified a large change over time (Figure 5.3) for CK (P = < 0.001, ηp^2 = 0.531) and hsCRP (P = 0.003, ηp^2 = 0.143), displayed in Figure 5.3. However, there were no group effects for CK (P = 0.488, ηp^2 = 0.054) or hsCRP (P = 0.318, ηp^2 = 0.084), nor any interaction effects for CK (P = 0.966, ηp^2 = 0.022) or hsCRP (P = 0.341, ηp^2 = 0.081).

Both CK and hsCRP values peaked at 24 hours post-run in the CH group, before returning to PRE at 48 hours (Figure 5.3).

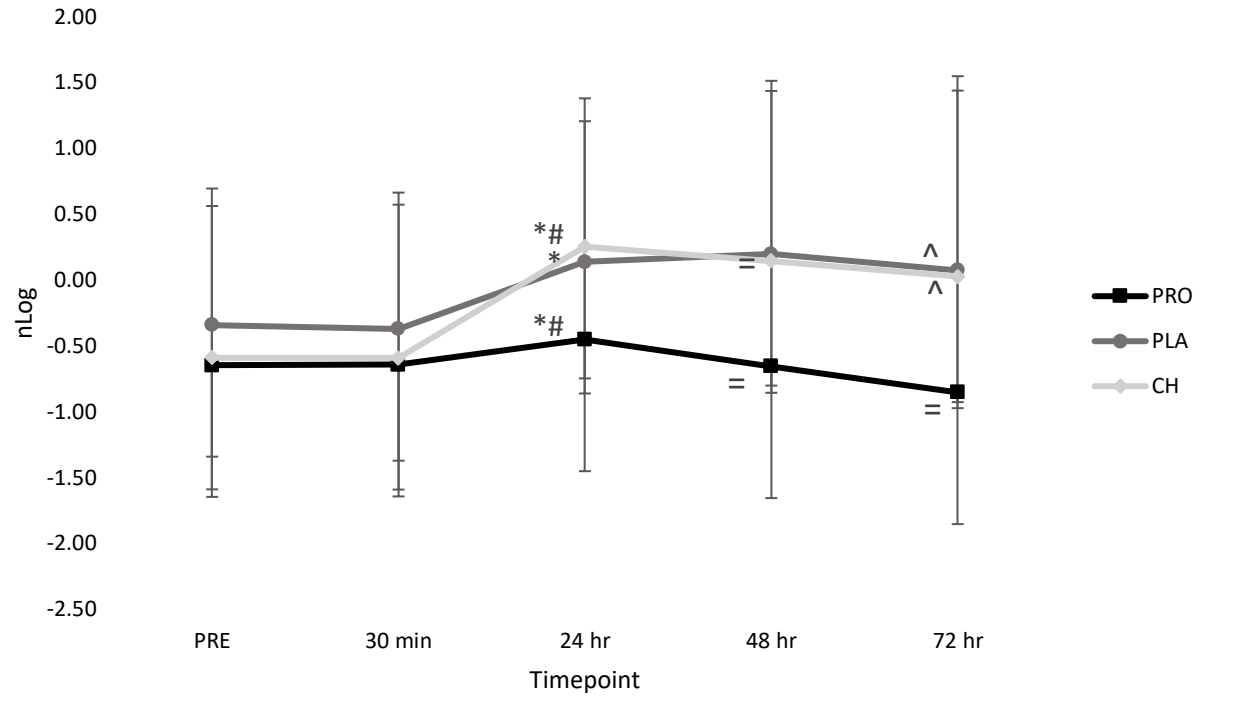
Figure 5.3

Changes in Muscle Damage and Inflammatory Biomarkers Following EIMD

a) *CK (nLog)*



b) *hsCRP (nLog)*



Note. Changes from pre-exercise (PRE) to post-exercise for milk protein (PRO), placebo (PLA), and collagen hydrolysate (CH) groups. Data are presented as means (SD). * Significantly different (*P* = < 0.05) from PRE, # significantly different to 30 min, = significantly different to 24 hr, ^ significantly different to 48 hr.

6. Discussion

6.1 Discussion Overview

EIMD has the potential to reduce exercise performance during subsequent bouts of exercise and/or cause a disruption to the normal daily activities of regular exercisers (Hody et al., 2019; Tesarz et al., 2012). Based on existing literature, dairy protein has been the 'typical' nutritional intervention used to minimise these problems and expedite acute recovery from EIMD (Robberechts et al., 2023). However, its effect on muscular function has been somewhat inconsistent despite its continued popularity, and only a limited number of studies have found an attenuation in DOMS (Draganidis et al., 2017; Cockburn et al., 2010; Hirose et al., 2013). Alternatively, there is emerging evidence to suggest that collagen can benefit recovery of multiple markers of EIMD, including DOMS (Clifford et al., 2019; Lopez et al., 2015), but few studies exist. Therefore, the aim of the study was to compare the effect of CH to PRO on acute recovery from EIMD, and subsequent DOMS, induced by downhill running in recreationally active males. It was hypothesised that 25 g of protein from CH would reduce ratings of DOMS, increase recovery for measures of muscular function, and attenuate biomarkers of muscle damage and inflammation following EIMD, to an equal or greater extent than an equivalent amount of protein from PRO, and that all protein sources would be more effective than a PLA.

The primary finding was that neither CH or PRO improved acute recovery from EIMD, and subsequent DOMS, following downhill running in recreationally active males. In contrast to our hypothesis, CH was not equal, nor more beneficial than PRO, and the protein sources were not better than a PLA. Both CH and PRO had no effect on measures of muscle soreness, muscular function, and biomarkers of muscle damage and inflammation at any timepoint of recovery. Nonetheless, all of the above indices of EIMD significantly changed over time as a result of 30 minutes of downhill running on a slope of -15% at a speed equivalent to 80% of predetermined VO_2max, suggesting that muscle damage had occurred.

6.2 Muscle Soreness

Thirty minutes of downhill running caused increased ratings of muscle soreness, suggesting that this protocol was an effective way to induce significant DOMS in the muscles of the lower body. Twenty-four hours post-EIMD, the highest muscle soreness rating was reported by the CH group during a squat. This response was within the typical timeline, where DOMS reaches a peak magnitude 24 to 48 hours after exercise (Contrò et al., 2016). It was also in line with a previous study by Southall-Edwards et al. (2022), who examined the effect of three different downhill running conditions on EIMD in recreationally active males. However, in contrast to this study that observed a full recovery in DOMS 48 hours post-exercise, we found significant ratings of muscle soreness in the CH group during a squat at the final timepoint of recovery. Although we observed a full recovery of DOMS during MVIC and squat for the other groups at 48 hours, DOMS may have persisted longer in the CH group due the variable and subjective nature of measuring pain.

While any modality of exercise that is strenuous, novel, repetitive, and/or involves eccentric contractions is capable of causing DOMS, downhill running is a mode that causes significant DOMS as our findings have demonstrated. Emerging a day or two after exercise, DOMS is a type of mechanically activated muscle soreness that is often described as a tender, dull, and/or achy pain sensation. Although it is acute, it may cause a disruption to normal daily activities that are otherwise easy to perform, which is problematic for anyone that regularly exercises (Tesarz et al., 2012). Researchers have spent a great deal of time and effort trying to understand DOMS, but it continues to be an ambiguous phenomenon that many do not fully understand (Cheshier & Jacobson, 2021; Mizumura & Taguchi, 2024). Further adding to the challenges researchers face, there is yet to be a nutritional intervention that effectively treats DOMS (Tanabe et al., 2022).

One intervention that has been used as a treatment for DOMS is dairy protein (Hirose et al., 2013). Its consumption might be of benefit by altering protein metabolism (Aussieker et al., 2023), however a limited number of studies have found evidence to suggest that it attenuates DOMS following EIMD (Cockburn et al., 2010; Draganidis et al., 2017; Hirose et al., 2013). In contrast, a large number of studies have failed to observe a benefit of dairy protein supplementation on DOMS recovery (Apweiler et al., 2019; Aussieker et al., 2023; Betts et al., 2009; Brown et al., 2018; Buckley et al., 2010; Cockburn et al., 2008; Dahlstrom Burnley et al., 2010; Davies et al., 2020; Eddens et al., 2017; Etheridge et al., 2008; Gee et al., 2019; Hilkens et al.,

2021; Nieman et al., 2020; Ormsbee et al., 2022; Robberechts et al., 2023; Saracino et al., 2020; ten Haaf et al., 2021; White et al., 2008). Similarly, we also found no effect of PRO on DOMS, at any time point of recovery, after the downhill run. Although some suggest a benefit, our finding was, perhaps, unsurprising. Not only was it in line with the majority of past research, but it also supports the assumption that the mechanistic actions of dairy protein do not primarily work to improve/minimise the underlying causes of DOMS.

The mechanistic actions of dairy protein primarily focus on the structural repair of muscle tissue damage, where the supply of essential amino acids from supplementation is believed to promote muscle protein synthesis (Aussieker et al., 2023). However, the popular view of structural muscle tissue damage (e.g., disrupted sarcomeres) is unlikely to be the primary cause of DOMS, but rather, it is more likely that damage to the extracellular matrix—an area densely innervated with nociceptors and free nerve endings, contributes to DOMS. (Wilke & Behringer, 2021). Therefore, due to dairy protein's lesser focus on the extracellular matrix (Holwerda & van Loon, 2022), we were unable to observe a reduction in DOMS at any timepoint of recovery in the PRO group.

Another intervention that has been used to alleviate DOMS following EIMD is collagen supplementation (Clifford et al., 2019; Prowting et al., 2021). In contrast to dairy protein, collagen's mechanistic actions may have more of an effect on the causes of DOMS by focusing on the extracellular matrix. Following damage, the delivery of non-essential amino acids glycine, proline, and hydroxyproline from ingested collagen are believed to enhance collagen synthesis, which may repair and/or replace damaged collagen fibrils in the matrix (Shaw et al., 2016). Twenty grams of collagen hydrolysate, consumed daily for seven days before and two days after 150 drop jumps, had a 'possible' benefit on DOMS at 24 hours and a 'likely' benefit at 48 hours post-exercise in recreationally active males (Clifford et al., 2019). Additionally, 3 g of collagen hydrolysate, consumed daily for six weeks prior to a retest of an upper body resistance challenge, led to a faster recovery of DOMS in recreationally active adults (Lopez et al., 2015).

Therefore, based on the existing literature, it was hypothesised that 25 g of protein from CH would reduce ratings of DOMS following EIMD, to an equal or greater extent than an equivalent amount of protein from PRO. However, in contrast to our hypothesis, CH had no effect on DOMS at any time point of recovery following the downhill run. Despite this finding being unexpected, similar studies have also failed to observe a benefit from collagen on exercise-induced DOMS (Aussieker et al., 2023; Prowting et al., 2021; Robberechts et al., 2023). Our

findings suggest that collagen supplementation is not an effective nutritional intervention for the treatment of DOMS following EIMD. However, it is possible that we did not observe a treatment effect because the timing of supplementation was after exercise. Previous studies reporting an attenuation in DOMS with collagen had supplemented it prior to EIMD (Clifford et al., 2019; Lopez et al., 2015). Although it must be noted, similar to Prowting et al. (2021), we also found it challenging to select appropriate timing for CH ingestion due to the sparse number of comparable collagen studies that are available. Therefore, it is possible that a benefit of CH on DOMS could still be obtained if it is consumed for a prolonged period before EIMD.

Another possible explanation for the ineffectiveness of CH on DOMS is the single measure we used to assess muscle soreness. In contrast to our study, Clifford et al. (2019) used two different measures of muscle soreness, VAS and PPT, to assess DOMS after 150 drop jumps. It might be likely that their use of multiple measures, which they proclaim to assess "different aspects of muscle soreness," could have increased their assessment's validity, and in turn, enabled them to observe a benefit of collagen on DOMS recovery (Clifford et al., 2019, p. 700). However, irrespective of this approach, they only found a positive effect based on decreased ratings of DOMS from VAS and not the PPT.

Alternatively, we may have failed to observe an effect of CH due to some validity issues surrounding the specific use of VAS. While it is a common way to measure DOMS following EIMD (Buckley et al., 2010; Clifford et al., 2019; Lopez et al., Prowting et al., 2021; Robberechts et al., 2023; White et al., 2008), it can only be used to rate the intensity of muscle soreness on a fixed scale and does not cover other aspects of DOMS (Cleather et al., 2007). Therefore, it is possible that our reliance on VAS caused us to miss other important aspects (e.g., the evoked emotions), and as a result, also the opportunity to observe a benefit of CH. However, according to Clifford et al. (2019), VAS is more valid than other, less subjective measures of muscle soreness, most notably the PPT. Nonetheless, regardless of the reason for the lack of effect, it remains difficult for researchers to measure a non-observable, subjective marker of EIMD.

6.3 Muscular Function

Overall, muscular function significantly reduced over time after the downhill run, with a peak loss of -16% at 30 minutes post-exercise that was followed by a return to baseline by the final timepoint of recovery. Individually, exercise performance reduced by -6% for CMJ, -8 % for

IMTP, -16% for MVIC, and a 6% increase in oxygen demand was observed for running economy. These findings, combined with significant increases in DOMS, demonstrate the effectiveness of using downhill running to induce EIMD in recreationally active males. Although our protocol was effective, the observed reduction in muscular function following downhill running contrasts that previously found by Etheridge et al. (2008). Despite using the same duration of exercise to induce damage in the same population to ours, they found that downhill running induced a smaller loss of -10% for MVIC and -8.7% for peak power output (PPO) at 48 hours post-exercise. These contrasting findings, however, might be attributed to the different downhill running intensities used to induce EIMD. In our study, we used a faster speed that was equivalent to 80% of pre-determined VO_2max on a steeper -15% slope, whereas Etheridge et al. (2008) used a slower speed that was equivalent to 75% of age predicted heart rate maximum on a smaller -10° slope. As a result, it is likely that the greater reduction in muscular function we observed was due to the higher intensity protocol.

The observed reduction in muscular function during the tests of running economy after downhill running also contrasts those reported by Braun and Dutto (2003), where a 30-minute downhill run at a speed equivalent to 70% of VO_2peak on a -10% slope led to a smaller increase in oxygen demand (3.2%). Although it is understandable that a less intense protocol will have less of an impact on oxygen demand during running economy, discrepancies are more likely due to differences in training history. As such, our participants were recreationally active; whereas those recruited by Braun and Dutto (2003) were well-trained endurance athletes. Therefore, it is highly likely that we observed a greater increase in oxygen demand because our participants had a lower amount of cardiovascular fitness.

The above findings highlight how EIMD can significantly reduce exercise performance during subsequent bouts of exercise in regular exercisers—a physiological and psychological deterrent to their future participation in exercise. However, our study is not the first to demonstrate the negative effects of EIMD on muscular function. It is a frequently stated problem in the existing literature and is often the motivation behind most nutritional interventions (Clifford et al., 2019; Buckley et al., 2010; Eddens et al., 2017).

Similarly applied to expedite acute recovery from DOMS, dairy protein may also be used to attenuate the loss of muscular function after EIMD. Previous research has suggested that the consumption of dairy protein enhances muscle protein synthesis and/or prevents muscle protein breakdown, which accelerates muscle repair and supports muscular function (Aussieker et al.,

2023; Tipton et al., 1999; West et al., 2017). Although some researchers question whether such mechanisms are actually responsible for the improvement in muscular function after EIMD (Pavis et al., 2021), previous applications of dairy protein have indeed been shown to benefit recovery (Buckley et al., 2010; Brown et al., 2018; Cockburn 2008; Cooke et al., 2010; Etheridge et al., 2008). This was demonstrated by West et al. (2017), who reported a moderate benefit from 25 g of whey protein, consumed immediately and the following morning after resistance exercise, on the CMJ performance of resistance-trained males. Moreover, a single 100 g of milk protein, consumed immediately after a 30-minute downhill run, improved the recovery of isometric force at 48 hours post-exercise better than a flavoured water PLA in recreationally active males (Etheridge et al., 2008).

However, on the same note, many studies have also failed to attenuate muscular function after EIMD with dairy protein (Appwelier et al., 2019; Betts et al., 2009; Dahlstrom Burnley et al., 2010; Davis et al., 2020; Eddens et al., 2017; Gee et al., 2019; Hilkens et al., 2021; Nieman et al., 2020; Ormsbee et al., 2020; Robberechts et al., 2023; Saracino et al., 2020; White et al., 2008). For example, one 40 g serve of casein protein (post-exercise) conferred no benefit to recovery of muscular function during one repetition maximum tests after a single bout of resistance exercise in resistance-trained males (Ormsbee et al., 2020). Furthermore, 20 g of whey protein hydrolysate (2 x daily for 4 days), immediately after simulated high-intensity road cycling and 100 drop jumps, failed to attenuate muscular function during MVIC and CMJ tests in well-trained male cyclists. Similarly, we also found 25 g of protein from PRO failed to improve recovery of muscular function during measures of exercise performance at all timepoints following the downhill run. Our findings, alongside those previously reporting no benefit, suggest that dairy-based protein is not an effective treatment for the loss of muscular function that occurs after EIMD. Therefore, it is perhaps surprising that it still remains one of the most recommended supplements for post-exercise recovery and that it is often viewed as a “prevailing nutritional practice” (Robberechts et al., 2023, p. 1), despite several inconsistent effects.

Nonetheless, dairy protein’s inconsistencies have prompted several researchers to explore alternative nutritional interventions, including collagen derived proteins. Corresponding to the mechanistic actions on how collagen may reduce DOMS, supplementation is thought to maintain or limit the loss of muscular function after EIMD by providing non-essential amino acids that boost rates of collagen synthesis, enhancing the repair of the extracellular matrix (Shaw et al., 2016). Certainly, supplementation has been demonstrated to support the recovery of muscular function

after EIMD (Lopez et al., 2015). Alongside a reduction in DOMS, Clifford et al. (2019) also found an improvement in CMJ performance 48 hours after drop jumps in participants that consumed 20 g a day of collagen hydrolysate. Likewise, Prowting et al. (2021) also observed a benefit to CMJ performance recovery at 24 hours post-exercise with 15 g· day of collagen hydrolysate, consumed for seven days before and five days after 100 drop jumps, in resistance trained males (Prowting et al., 2021).

Consequently, based on dairy protein's inconsistent effect(s) on muscular function recovery and the emerging research on collagen, it was hypothesised that 25 g of protein from CH would increase recovery for measures of muscular function following EIMD, to an equal, if not greater extent than an equivalent amount of protein from PRO. However, in contrast to our hypothesis and previous research, CH was not beneficial for recovery of any measure of muscular function at any timepoint following the downhill run. This suggests that collagen supplementation is not a useful nutritional intervention for expediting recovery of muscular function following EIMD. Although the existing literature on collagen in the context of EIMD is limited and it is difficult to find studies with similar results, these findings partially support those reported by Robberechts et al. (2023). They found 20 g of collagen hydrolysate combined with 25 g of whey protein, consumed during a 3-week training period, was not better at improving muscular function recovery than 45 g of whey protein alone in young fit males. While their study similarly found that collagen (combined with whey protein) did not surpass the effectiveness of dairy protein, there are some key differences between their study and ours. Firstly, our study compared all protein sources to a PLA. We also separated both collagen and dairy protein supplements. In contrast, Robberechts et al. (2023) did not use a comparative PLA and they combined collagen with a portion of dairy protein. Furthermore, we induced EIMD using a single bout of downhill running in recreationally trained males, whereas they used a pre-test at the beginning and a post-test at the end of a 3-week period of resistance training in young fit males. Therefore, due to several differences in study design, it is difficult to directly compare our findings to those previously reported by Robberechts et al. (2023).

Although it might be difficult to identify and explain the precise reason(s) as to why we were not able to observe an effect of CH supplementation, as well as PRO, on recovery of muscular function following EIMD, a possible explanation is our participants' high intake of daily dietary protein. It has been well established that protein supplementation has a minimal benefit to individuals consuming a daily amount of dietary protein that is already sufficient to meet their

recovery needs (Davis et al., 2020). Reporting findings similar to ours, Apweiler et al. (2018) found that 40 g of pre-sleep casein protein failed to benefit recovery of muscular function after 100 drop jumps (performed in the morning) in active participants that already consumed ~1.5 g· kg· day of dietary protein. Therefore, it is highly possible that our recreationally active participants already consumed enough dietary protein to improve muscular function recovery after EIMD, without the need for 25 g of supplemental protein from CH or PRO.

Another feasible explanation for the lack of effect on muscular function might relate to the relatively short duration of protein supplementation. Although, single doses of dairy protein have been previously shown to benefit recovery (Buckley et al., 2010; Cockburn et al., 2008; Cockburn et al., 2010; Etheridge et al., 2008), a longer duration of supplementation might be required, particularly in regards to collagen. Those previously reporting a positive effect of collagen on recovery of muscular function after EIMD have all used durations longer than that used in the current study (Clifford et al., 2019; Lopez et al., 2015; Prowting et al., 2021). Some researchers have recommended that it be consumed for a minimum of three months for any substantial benefit (Khatri et al., 2022). However, limited dosage recommendations for collagen exist in the context of EIMD (Clifford et al., 2019). Therefore, it remains possible that both protein supplements, most notably collagen, might benefit recovery of muscular function following EIMD but only when they are consumed over an extended period of time.

6.4 Biomarkers

6.4.1 Muscle Damage

The downhill run caused large changes over time for CK, with a peak increase of 933 U/L at 24 hours that was followed by a return to baseline at 48 hours post-exercise. This finding suggests that 30 minutes of downhill running is a successful way to induce significant muscle damage. It also partly corresponds to that found by a previous study by Philpott et al. (2023); although their selected intensity somewhat differed, who found a 40-minute downhill run on a -10% slope at 70% of VO_2peak caused CK to increase to 763 (158) U/L at 24 hours post-exercise in male team sport players.

Some existing research suggests that dairy protein's recovery benefits are largely based on its effects on muscle repair (Aussieker et al., 2023). It is understood that supplementation may

promote several muscular recovery mechanisms (e.g., muscle protein synthesis or boost satellite cell activity) following EIMD, which may help to maintain membrane integrity and reduce the leakage of intramuscular proteins into circulation (Reitelseder et al., 2014). However, although some studies have demonstrated an attenuation in muscle damage biomarkers after EIMD (Brown et al., 2018; Cockburn et al., 2010; Hirose et al., 2013; Nieman et al., 2020), the majority of research suggests otherwise (Betts et al., 2009; Buckley 2010; Cooke et al., 2010; Davies et al., 2020; Draganidis et al., 2017; Eddens et al., 2017; Hilkens et al., 2021; Ormsbee et al., 2022; Saracino et al., 2020; ten Haaf et al., 2021; White et al., 2008). For instance, 0.4 g· kg· day of whey protein, consumed for three days after eccentric exercise, had no effect on CK in untrained males (Dahlstrom Burnley et al., 2010). Moreover, 1.5 g· kg· day of whey protein isolate, consumed for 14 days post-exercise, did not attenuate CK, nor LDH, after untrained males performed a bout of resistance exercise (incl. leg press, extension & flexion; Cooke et al., 2010). Consistent with the majority of research, we also found that 25 g of protein from PRO failed to attenuate the efflux of CK, at all timepoints of recovery, after 30 minutes of downhill running. As a result, this outcome does not support the use of dairy protein for improving recovery of muscle damage induced by exercise.

Alternatively, research has also proposed that collagen supplementation might attenuate muscle damage and the leakage of intramuscular proteins after EIMD by enhancing the remodelling of the extracellular matrix, which supports the structural integrity of the muscle membrane (Prowting et al., 2021); although there is some uncertainty around whether this benefit is due to the remodelling of connective tissue and/or repair of muscle fibres (Bischof et al., 2024). Nonetheless, combined with other beneficial effects, Lopez et al. (2015) also found 3 g· day of collagen hydrolysate, over a six week period prior to a retest of an upper body resistance challenge, attenuated the post-exercise increase of CK and LDH in recreationally active adults (Lopez et al., 2015). Furthermore, 15 g· day of collagen (mix of specific peptides), consumed for 12 weeks during concurrent exercise, minimised the increase of CK, LDH, and Mb after a post-test involving 150 drop jumps in sedentary to moderately active males (Bischof et al., 2024). Therefore, we also expected to observe a similar effect of collagen supplementation. However, opposing our hypothesis, CH did not attenuate the increase of CK at any timepoint, equivalently, nor more effectively than a protein-matched PRO after the downhill run in recreationally active males. Likewise, Clifford et al. (2019), despite an improvement in DOMS and CMJ performance recovery,

also found collagen hydrolysate (20 g· day for 9 days) failed to attenuate LDH and CK after recreationally active males performed drop jumps.

While our findings suggest that CH, similar to PRO, is not a useful recovery intervention for muscle damage induced by exercise, it is possible that inter-individual variability masked any treatment effect(s). Indeed, CK is a highly variable biomarker of muscle damage, and high or low responses often make subtle group differences more challenging to detect (Baird et al., 2012). Similarly, existing literature has also encountered the same issue, where CK variability has been used to explain discrepant findings (Clifford et al., 2019; White et al., 2008). Therefore, it was possible that this contributed to CH and PRO having no effect on systemic muscle damage after EIMD in our study. However, it is more likely that neither source of protein repairs the damaged sarcolemma or stops secondary damage from neutrophils or other pro-inflammatory cells. Nonetheless, we cannot rule out a reduction in localised muscle damage following supplementation of CH or PRO.

6.4.2 Inflammation

Combined with a significant increase in CK, hsCRP also displayed a significant change over time post-exercise. Thirty minutes of downhill running induced a peak increase of 1.29 mg/L at 24 hours, which was followed by a return to baseline at 48 hours post-exercise. This finding suggests that downhill running is a strenuous enough exercise to trigger an inflammatory response. However, it contrasts the results reported by Philpott et al. (2023), who reported that hsCRP did not significantly increase above baseline after a 40-minute downhill run. While the protocol choosen by Philpott et al. (2023) was a longer duration than ours, inconsistencies in the inflammatory response might be attributed to differences in protocol intensity or participant training history. Our protocol had participants running on a steeper slope (-10% vs. -15%) at a higher speed (70% VO_2peak vs. 80% VO_2max). We also recruited recreationally active males that were between 18 to 40 years old, whereas Philpott et al. (2023) recruited young male athletes that regularly participated in exercise. Therefore, either due to the more intense protocol or that regular exercise has been shown to blunt exercise-induced inflammation (Ormsbee et al., 2022), this enabled us to trigger an inflammatory response using downhill running.

Dairy protein hydrolysates, such as whey protein, contain individual bioactive fractions that have been suggested to exert anti-inflammatory effects on the body (Auestad & Layman, 2021).

This may include an attenuation in pro-inflammatory cytokines, enzymes, and other pathways that upregulate the inflammatory response following supplementation (Da Silva et al., 2017). However, in the context of exercise-induced inflammation, dairy protein has often failed to exert such effects (Betts et al., 2009; Buckley et al., 2010; Draganidis et al., 2017; Eddens et al., 2017; Hilkens et al., 2021; Nieman et al., 2020; Ormsbee et al., 2022; Robberechts et al., 2023; Saracino et al., 2020). Similarly, 25 g of protein from PRO had no benefit on inflammation triggered by a 30-minute downhill run, based on post-exercise changes in hsCRP, in the current study. Although PRO contains a smaller percent of whey protein; possibly not enough to have an effect on inflammation, it is more likely that dairy protein does not reduce inflammation after EIMD.

Alternatively, the consumption of hydrolysed collagen may have an anti-inflammatory effect (Sivaraman et al., 2021). It has been shown to attenuate cytokine release and downregulate the inflammatory response in the context of skin and beauty (Brandao-Rangel et al., 2022). Furthermore, in contrast to dairy protein, it has also been demonstrated to minimise inflammation after muscle-damaging exercise (Lopez et al., 2015). For instance, despite insisting that collagen supplementation had little anti-inflammatory effect, Clifford et al. (2019), observed a moderate decrease in IL-6 at 1.5 hours after drop jumps. However, based on hsCRP, we found that CH had no effect on inflammation triggered by the downhill run, which indicates that collagen does not reduce the inflammatory response after EIMD. This finding is in line with a previous study by Bischof et al. (2024), who found that 15 g· day of collagen (mix of specific peptides), over 12 weeks during concurrent exercise, did not attenuate hsCRP, nor IL-6, when sedentary to moderately active males performed 150 drop jumps. A possible explanation also proposed by Bischof et al. (2024), is that CH, as well as PRO, failed to exert a post-exercise anti-inflammatory effect due to significant hsCRP variability, which may have made it harder to detect subtle group differences. Nonetheless, in the absence of muscle biopsies, we cannot rule out a reduction in localised inflammation with CH or PRO supplementation.

6.5 Strengths, Limitations, and Recommendations

One strength of our study was the type of protocol we used to induce EIMD. The 30-minute downhill run at 80% of predetermined VO_2max speed on a -15% slope caused a large change over time in ratings of DOMS, measures of muscular function, and biomarkers of muscle damage and inflammation, which was a strong indication that EIMD had occurred in our

recreationally trained male participants. It is imperative that researchers successfully induce EIMD, otherwise there is less of a need for nutritional intervention/smaller window of opportunity for supplementation to benefit recovery (Eddens et al., 2017). However, many have contrasting views on how to go about inducing EIMD, and subsequent DOMS. Some researchers have used drop jumps (Apweiler et al., 2019; Bischof et al., 2024), while others have used resistance (Cooke et al., 2010; Gee et al., 2019) or endurance exercise (ten Haaf et al., 2021). Indeed, this demonstrates how to induce damage using a wide range of exercise, but it also highlights a lack of consistency across different studies and suggests that any exercise can be used to induce EIMD, validated or not. However, this is a flawed assumption; not all exercise causes EIMD and as a result, not all protocols successfully induce EIMD (Ormsbee et al., 2022; ten Haaf et al., 2021). Nonetheless, our findings support the use of downhill running for the induction of EIMD.

Another strength of our study was the use of an isoenergetic PLA. This ensured that all participants, regardless of their assigned group, received an equal amount of energy, and that the additional energy from the protein supplements would not explain any possible group differences. The use of an isoenergetic PLA was recommended by a previous study by Cockburn et al. (2010) after they found that the intervention group received more energy than the water PLA group. As a result, they could not rule out the possibility of this having an effect on recovery. While we were not able to observe an effect of dairy protein or collagen, if we were to however, we would have been able to attribute any benefit(s) in greater confidence to the intervention(s) used to expedite recovery from EIMD.

Our comprehensive assessment of EIMD was also another strength of our study. Not only did we assess DOMS, but we also assessed muscular function using four different exercise tests, combined with biomarker analysis. Due to the complex nature of EIMD, including variable symptom presentation and recovery (Allen et al., 2004), it is imperative that researchers use multiple measures in their assessment of EIMD. Deciding against assessing some of the main symptoms (DOMS or muscular function) for instance; which many studies have done (Aussieker et al., 2023; Bischof et al., 2024; Cooke et al., 2010; ten Haaf et al., 2021; West et al., 2017), would not capture the full effect(s) of an intervention and make it more difficult to determine whether it does or does not improve EIMD recovery.

Although our choice of EIMD protocol, use of an isoenergetic PLA, and comprehensive assessment of EIMD were strengths of our study, it was not without its limitations. Firstly, the main limitation was a lack of dietary control regarding the daily dietary intake of protein, most

notably during the 3-day study period. Although 24-hour diet records were used to determine how much protein each participant normally consumed, along with verbal instructions to maintain this for the duration of the study, we did not implement any other form of dietary control. Therefore, it was possible that participants may have deviated from what was first stated in their 24-hour records and consumed a higher or lower amount of daily protein, which consequently could have caused PRO or CH supplementation to be ineffective. Nonetheless, a lack of dietary control is a shared limitation that is frequently mentioned in the existing literature (Apweiler et al., 2019; Cockburn et al., 2008; Dahlstrom Burnley et al., 2010; Eddens et al., 2017; Gee et al., 2019; Nieman et al., 2020; Ormsbee et al., 2022). Some studies have recognised this widespread issue and attempted to minimise it by standardising diets and catering all meals during the study period (Davies et al., 2020; Saracino et al., 2020). While this approach might be a little excessive, not to mention expensive (financial constraints limited our ability to employ such control), at bare minimum future studies would benefit from employing a moderate level of control by using diet records during the study period. This would help to minimise the influence of dietary confounders, including an excessive or insufficient daily dietary intake of protein.

However, even if we employed a greater amount of dietary control and our participants did not consume any additional dietary protein (than what was first recorded), it was highly likely that their intakes were already disproportionately high for recreationally active people. For their inclusion into the study, the dietary protein cut-off was 1.6 g· kg· day. While all participants were below this amount prior to protein supplementation, after supplementation they would have been over; consuming double the daily recommended amount of protein for general population (0.8 g· kg· day; Master & Macedo, 2020)—an amount of protein that likely exceeded their recovery needs. However, it is common for recreationally trained people to consume high amounts of protein (as ours findings demonstrate), which brings into question the a need to use a supplement to recover at all. Nonetheless, future research should consider the sampled populations' daily dietary intake of protein and how it can become a limiting factor during experimental protein supplementation.

Another limitation of our study was that we relied on indirect measures to assess EIMD. Despite our assessment being comprehensive, in line with the existing literature (Clifford et al., 2019; Eddens et al., 2017; Prowting et al., 2021, White et al., 2008), and not limiting our ability to accurately assess muscular function (exercise tests are a valid measure of muscular function; Chalchat et al., 2022), it may have somewhat restricted our ability to assess muscle damage and

inflammation. Firstly, muscle damage and inflammatory biomarkers predominately indicate systemic changes following EIMD (Allen et al., 2004). Therefore, it was possible that supplementation could have had a localised effect that we were unable to detect. Furthermore, the biomarker we used to assess muscle damage (CK) is highly variable (Cooke et al., Ormsbee et al., 2022; Prowting et al., 2021; White et al., 2008), and high or low responses may have made it difficult for us to observe change after supplementation. Previous studies have also expressed similar difficulties with biomarker analysis and have recommended that future studies should perform muscle biopsies (Clifford et al., 2019; Prowting et al., 2021). However, financial and technical constraints often make them unfeasible, not to mention the procedure is more invasive. Alternatively, as systemic elevations remain an accepted measure in the field, another recommendation is that future studies examine post-exercise changes in less variable biomarkers.

Our study also did not measure any underlying action(s) of both protein supplements. Whist we could have used biomarkers to assess their actions, including collagen synthesis or breakdown, we ultimately chose not to as previous studies have often been unable to detect a meaningful change in biomarkers after supplementation, particularly with collagen (Clifford et al., 2019; Prowting et al., 2021: Robberechts et al., 2023). However, we do not entirely view this to be a limitation as we were examining the effect(s) of two protein supplements, not the necessarily the mechanisms. Although if were to observe a benefit from CH or PRO, it would have reduced our ability to draw any meaningful conclusions about what actions were responsible. Therefore, it would be wise that future studies be equipped with the ability to explain any effect(s) if they happen to occur, be that through biomarker analysis or again through muscle biopsies.

All our participants were ‘free-living’ recreationally active male volunteers. Accordingly, alongside no control over their daily dietary intake of protein during the study period, there was also no control over their physical activity. While we asked participants to abstain from all strenuous exercise outside of what was prescribed and normal walking (two days before and during the study period), similar to the suggestions made by Aussieker et al. (2023), Brown et al. (2018), and Dahlstrom Burnley et al. (2010), we cannot be certain whether these instructions were followed. Therefore, it was a possibility that our participants may have engaged in other exercise that further compounded the muscle damage induced by the downhill run, and as a result, this could have interfered with the effects of supplementation. However, physical activity logs or questionnaires would have been an easily implemented method of control that would have reduced any unprescribed exercise.

Finally, the characteristics of our chosen population sample (healthy recreationally active males, between 18 to 40 years old), the type of EIMD protocol used (downhill running on a treadmill), and the controlled environment it was induced in (laboratory), mean we are careful not to generalise our findings to other populations, particularly females and athletes for example. It is worth noting that we chose to only include males in our study, as there has been evidence to suggest that the menstrual cycle affects the extent of EIMD that occurs (Romero-Parra et al., 2021). Nonetheless, it would be worthwhile for future research to extend research in this area to include different populations and sporting contexts.

7. Conclusion

EIMD is a transient phenomenon caused by intense, repetitive, new, and/or eccentric exercise (Allen et al., 2004). Despite its short-lived nature, it may cause problems for regular exercisers by impairing exercise performance during subsequent bouts of exercise and/or by causing a disruption to regular daily activities that are otherwise easy to perform (Hody et al., 2019; Tesarz et al., 2012). Much of the existing literature on nutritional interventions used to treat recovery from EIMD has primarily focused on dairy-based protein supplements (Etheridge et al., 2008; Howatson & van Someren, 2008). The reason for this might be due to dairy protein's positive effect on muscle protein synthesis and growth, when used in conjunction with resistance exercise (Master & Macedo, 2021). However, the effect of dairy protein in the context of EIMD has been less than consistent. Alternatively, the effect of other sources of dietary protein, including collagen—an underutilised by-product created by the meat industry, has been recently examined (Bischof et al., 2024). Despite some promising results, such as attenuated DOMS, improved exercise performance, and/or an alteration in biomarkers of muscle damage and inflammation (Clifford et al., 2019; Lopez et al., 2015; Prowting et al., 2021), few studies have investigated collagen directly to dairy protein (Aussieker et al., 2023; Robberechts et al., 2023). Therefore, using a PLA-controlled, double-blinded, independent group study design, we endeavoured to compare the effect of CH to PRO on acute recovery from EIMD, and subsequent DOMS, induced by downhill running in thirty-three healthy active males. We hypothesised that 25 g of protein from CH would reduce ratings of DOMS, increase recovery for measures of muscular function, and attenuate biomarkers of muscle damage and inflammation following EIMD, to an equal or greater extent than an equivalent amount of protein from PRO, and that all protein sources would be more effective than a PLA. Indeed, we were able to demonstrate how 30 minutes of downhill running at 80% of previously determined VO_2max speed on a -15% slope can induce significant EIMD in recreationally active males, however, while past research has found evidence to suggest that collagen supplementation is beneficial for recovery from EIMD (Clifford et al., 2015; Lopez et al., 2015; Prowting et al., 2021), the current study did not find evidence to support this claim. Conversely, CH did not alter any marker of EIMD, at any timepoint of recovery, equal to or better than PRO. Furthermore, both protein sources were not more effective at improving recovery from

EIMD than a PLA. These three conclusions have some important practical implications, not only for researchers but also for exercise practitioners, who advise regular exercisers on ways to minimise EIMD. Firstly, we have shown that downhill running is an effective way to induce EIMD in a controlled laboratory environment. Secondly, it is likely that collagen is not an effective nutritional intervention for expediting acute recovery from EIMD and subsequent DOMS. Finally, as both protein sources were ineffective, it is likely that dairy protein is also unsuitable. While our study was not able to find an answer, it is hoped that through our contribution to the existing literature on the effects of collagen in the context of EIMD, we can support future studies to answer the following question “what is the best nutritional intervention for expediting acute recovery from EIMD.”

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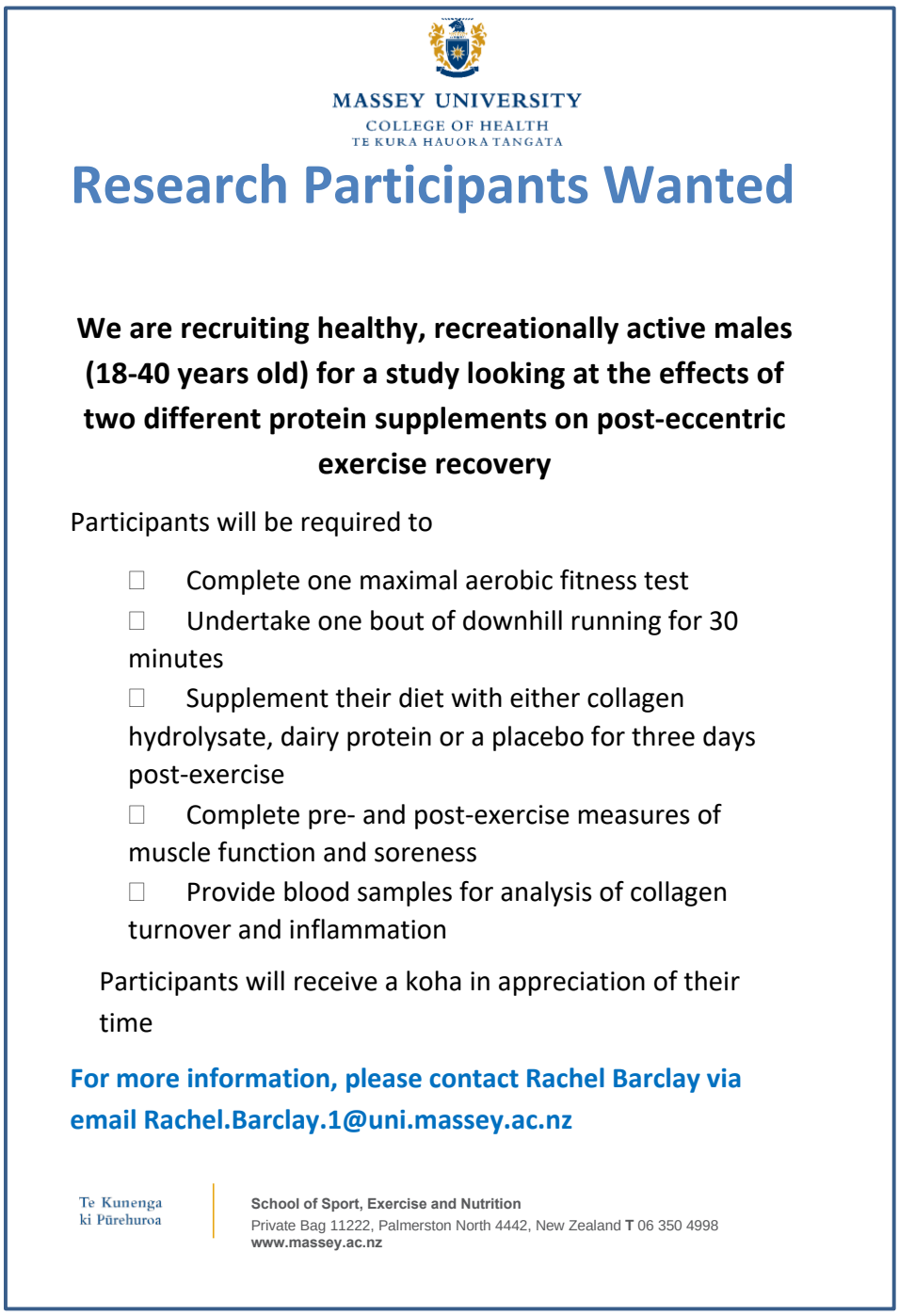
Appendices

Appendix A: Participant Advertisement



MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Research Participants Wanted

We are recruiting healthy, recreationally active males (18-40 years old) for a study looking at the effects of two different protein supplements on post-eccentric exercise recovery

Participants will be required to

- Complete one maximal aerobic fitness test
- Undertake one bout of downhill running for 30 minutes
- Supplement their diet with either collagen hydrolysate, dairy protein or a placebo for three days post-exercise
- Complete pre- and post-exercise measures of muscle function and soreness
- Provide blood samples for analysis of collagen turnover and inflammation

Participants will receive a koha in appreciation of their time

For more information, please contact Rachel Barclay via email Rachel.Barclay.1@uni.massey.ac.nz

Te Kunenga ki Pūrehuroa

School of Sport, Exercise and Nutrition
Private Bag 11222, Palmerston North 4442, New Zealand **T** 06 350 4998
www.massey.ac.nz

Appendix B: Participant Information Sheet and Consent Form

Participant Information Sheet

Effect of collagen hydrolysate on recovery from downhill running

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Sponsor: School of Sport, Exercise and Nutrition, Massey University

Lead Researcher: Dr Matthew Barnes

Study Site: Human Performance Laboratory.

Massey University,

Palmerston North

Contact phone number: 06 9516822

Ethics committee ref.: EXP12330

You are invited to take part in a study investigating the effect of collagen hydrolysate on recovery from downhill running. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. Before you decide, you may want to talk about the study with other people such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is 11 pages long, including the Consent Form. Please make sure you have read and understood all the pages.

Voluntary Participation and Withdrawal From This Study

Participation in this study is voluntary. You are free to decline to participate and may withdraw from the research at any practicable time, without experiencing any disadvantage.

What is the purpose of the study?

Unaccustomed or strenuous exercise can result in muscle soreness, inflammation and decreased muscle function, which may negatively impact subsequent exercise performance and activities of daily life. As such, a significant amount of research has been carried out to

find ways of alleviating the symptoms of exercise induced muscle damage (EIMD) and speeding up repair, including the use of various dietary strategies.

Supplementation with dairy protein has shown promise as a method of reducing the symptoms of EIMD, and emerging evidence indicates that hydrolysed collagen preparations are also valuable in exercise recovery by decreasing delayed onset of muscle soreness (DOMS). Despite the growing evidence, there is currently no consensus as to the effect of protein or collagen supplementation on recovery from EIMD.

Therefore, the aim of this study is to investigate the effects of collagen hydrolysate on recovery from EIMD and its ability to reduce DOMS. We hypothesise that, when compared to an equivalent amount of dairy protein or a placebo, collagen hydrolysate will reduce muscle soreness and inflammation and increase the rate of recovery for measures of muscle function.

HOW IS THE STUDY DESIGNED?

We are recruiting 42 healthy, recreationally active males aged between 18 and 40 years old. If you decide to participate in this study, you will be asked to visit the Human Performance Lab, Massey University on 5 occasions, for a total of 6.5 hours. You will be randomly allocated into one of three groups (collagen hydrolysate (CH); dairy protein (DP); placebo (PL)). As this study is double blinded, neither you nor the investigators will know which treatment you are on until the study has been completed.

To test the effect of collagen hydrolysate on muscle recovery, you will ingest a supplement containing either CH, DP or PL 45 minutes after completing 30 minutes of downhill running exercise and again 24, 48 and 72 hours later. CH and DP will contain 25 g of protein, flavouring and maltodextrin, PL will contain flavouring and maltodextrin and will be consumed as a powder that can be added to water.

You will be provided with a standardised drink to consume 2 hours before your first visit. At 24, 48 and 72 hours you will consume the supplement provided.

Before, immediately after and 24, 48 and 72 hours after the downhill run, your muscle function, muscle soreness and running economy will be measured. Additionally, 16mL blood samples will be taken by a trained phlebotomist from a vein in your arm at these times.

The study protocol and timeline of measures are outlined in Figure 1 and Table 1.

WHO CAN TAKE PART IN THE STUDY?

If you are a healthy male aged between 18 and 40 years old, you may take part in this study. To ensure your safety and the quality of the data, you will be excluded from the study if you meet any of these exclusion criteria:

- You undertake regular (more than 4 times per week) strenuous exercise; this includes heavy resistance exercise or long duration/high intensity running.
- You are dairy/lactose intolerant (the protein powder is dairy based)
- You are vegetarian or vegan (as the supplements are made from animal products).

- You consume a high protein diet (more than 1.6 grams of protein per kg of bodyweight per day) and/or you regularly consume protein supplements.
- You regularly use ergogenic supplements such as pre-workout products, branched chain amino acid (BCAA) powder, creatine monohydrate.
- You have an injury or medical condition that could be made worse by exercising or stops you from participating in high intensity exercise
- You have been ill in the 14 days before the study begins
- You are participating in another research trial

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

Health Screening and Familiarisation

If, after reading this Participant Information Sheet, you want to participate in the study you will be invited to the laboratory for health screening and familiarisation (Visit 1). Prior to this visit, you will be asked to fill out a 24-hour diet diary and return that to us so that we can assess your normal dietary protein intake. One of the research team will call you to go through the diet dairy with you to ensure we get an accurate assessment of your protein intake. During Visit 1 you will be given an opportunity to ask any questions you have about the study, and you will fill out a health questionnaire to assess your eligibility for inclusion. No one outside the research team will see your health questionnaire and it will be destroyed at the end of the study or returned to you if requested.

If you are eligible for inclusion in the study, we will proceed with the familiarisation process, which will get you used to the room, equipment and exercise that will be used during the trials. A member of the research team will explain what the familiarisation session will involve and answer any questions or concerns that you have. After signing the study consent form, your weight, height, and resting blood pressure will be measured. We will then explain how the muscle soreness questionnaire works, before taking you through each of the tests being used in the study, as outlined below.

You will then undertake a graded exercise test on a treadmill to assess your cardiovascular fitness (VO_2max) so that we can identify the speed used for the downhill running protocol and running economy test. This test involves you running continuously on a treadmill at four increasingly faster, submaximal speeds. Each speed will be maintained for 4 minutes. Once the last submaximal speed is reached, the speed of the treadmill will be increased every 1 minute. You will be asked to continue the test until you think that you can only run for one more minute. During the test, you will wear a silicone face mask over your mouth and nose, that is connected to a plastic tube, so that expired air can be collected and analysed. We will also ask you to wear a heart rate monitor so we can monitor your heart rate during exercise.

You will then be given 10 minutes recovery before completing 5 minutes of downhill running, to familiarise you with this form of exercise. At the end of your exercise, a member of the research team will be available to answer any further questions you have. They will then arrange a date for you to return to the laboratory to start the trial.

This familiarisation visit will take approximately 1.5 hours.

From 48 hours before the trial until the last measures are made 72 hours post-downhill run, you will be asked to refrain from exercise (apart from normal walking), consuming alcohol,

anti-inflammatory medications, recreational drugs and sport supplements, including protein supplements. Additionally, over this time, you will be asked to record your daily food intake in a diet diary (to be analysed for energy and macronutrient content).

Please note that you should call the Principal Investigator ahead of attending any session if you, or anyone you have close personal contact with have COVID-19, or are displaying symptoms of any transmissible illness.

Trial visits

Pre-trial: A member of the research team will be in contact with you two days before the start of your main trial visit to confirm your participation. They will remind you of what you should avoid before visiting the lab, as outlined above.

Main trial day (Visit 2): You will be provided with a standardised meal and volume of water to consume 2 hours before returning to the laboratory for baseline testing. Measures of muscle soreness, muscle function and running economy will be made and a blood sample will be collected at this time. After resting for 15 minutes, you will then complete 30 minutes of downhill running at -15% incline at 80% of your VO_2max speed.

30 minutes after completing the downhill run, you will repeat the baseline measures and then consume your first drink.

This main visit will take approximately 2 hours.

Follow up visits (Visits 3, 4 and 5): Two hours before each follow up visit, at 24, 48 and 72 hours after the downhill run, you will consume the standardised meal, water and the supplement. You will then return to the laboratory and measures of muscle soreness, muscle function, running economy and blood sampling will be carried out (as done at baseline).

These follow up visits will take approximately 1 hour.

The total time commitment for this study is approximately 6.5 hours, excluding your travel time to and from the laboratory.

Measures

Muscle soreness:

You will rate how sore the muscles of your lower body are on a scale of 0 to 10, with 0 being no soreness and 10 being extreme soreness.

Muscle function:

Lower body strength will be measured using a test called the midthigh pull. This involves standing on a small platform, with your knees and hips bent and a bar held in your hands. The bar is connected to a load cell and the platform by a chain; the load cell will measure how much force you can produce. You will be instructed to pull against the bar as forcefully as possible for 3 seconds; this will be repeated 3 times with a 30 second rest between each effort.

To test the strength of the quadriceps (thigh) muscles of your right leg, you will be seated on a machine called an isokinetic dynamometer. You will be strapped in place before completing 3 maximal effort leg extensions, each effort will be separated by 30 seconds rest.

Your lower body power output will be measured using a verical jump test. Jump height will be measured using a digital jump mat. You will stand on the mat, place your hands on your hips and, using a counter movement, jump as high as possible. You will make three attempts at this test, with a 30 second rest between jumps.

The amount of oxygen you use and effort required to run at a set speed (running economy) will be measured by running on a treadmill. A heart rate monitor will be fitted around your chest and you will then undertake a 5 minute warmup at 60% VO_2max speed. You will then complete 5 minutes running at 80% VO_2max speed with a silicone face mask fitted over your mouth and nose so that expired gases can be measured.

Blood sampling: Blood will be collected from a vein in your arm by a trained and experienced phlebotomist. This will involve placing a tourniquet around your upper arm, cleaning the insertion site with an alcohol swab and then insertion of a small needle into the vein. 24mL of blood will be collected into vacutainer (blood collection) tubes.

Figure 1. Study protocol.

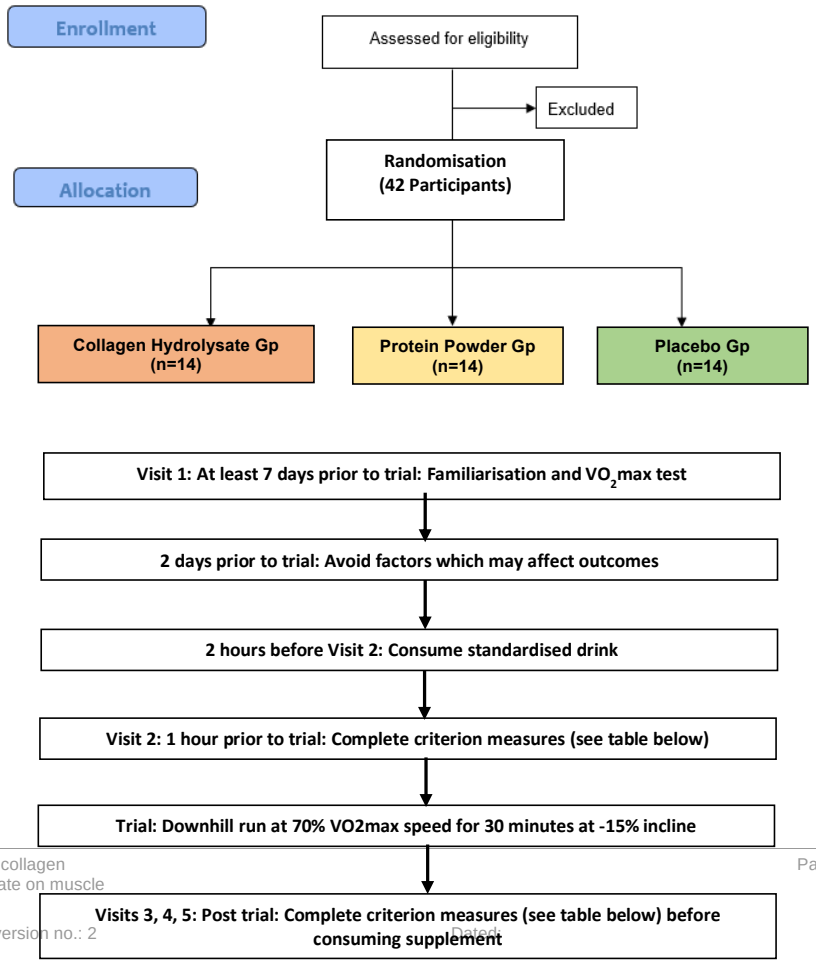


Table 1. Timeline of measures used to assess the effects of collagen hydrolysate on muscle recovery.

	Time										
	-2h	-1h	0h	30min	45min	22h	24h	46h	48h	70h	72h
Standardised drink	X										
Supplement drink					X	X		X		X	
Blood sample		X		X			X		X		X
Vertical jump		X		X			X		X		X
Mid-thigh pull force		X		X			X		X		X
Knee extension force		X		X			X		X		X
Running economy		X		X			X		X		X
Downhill run			X								

WHAT WILL HAPPEN TO MY BLOOD SAMPLES?

Blood samples will be collected in vacutainer tubes, centrifuged and the serum/plasma removed and frozen at -80˚C until analysis. Analysis will be carried out at a later date, after which samples will be disposed of. Samples will be analysed for biomarkers of collagen synthesis and inflammation.

You have the right to request the return of your samples once they have been analysed. The sample provided remains your property and *taonga* throughout the entire research process and you have the right to have the sample returned after analysis.

You have the right to request an act of *tikanga* in any form that acknowledges the *tapu* nature of your sample i.e., *karakia* (blessing), *whakatau* (introduction) and *tautoko* (support, usually in the form of *whānau* (family) members) and any other process of *tikanga* that you may require.

Cultural considerations: You may hold beliefs about sacred and shared values about your tissue samples and/or data originating from this tissue. The cultural issues associated with storing your tissue and data should be discussed with your family/whanau as appropriate. If you need cultural support this can be provided. Please let us know and we will arrange this for you, or you can ring the number at the bottom of this Participant Information Sheet and Consent Form. We respect the importance of these values and beliefs so please inform us if you wish to have whanau support present.

WHAT ARE THE POSSIBLE RISKS OF THIS STUDY?

Exercise:
Because of the maximal intensity of the graded exercise test (VO_2max test), you are likely to feel fatigue and discomfort; this is quite normal and these sensations will pass relatively quickly once exercise stops. Additionally, as with any exercise, there is a small risk of musculoskeletal injury.

To reduce any feelings of discomfort and the chance of injury, during the graded exercise test, speed and incline are increased slowly providing a warm-up period at the start of the test. An active cool-down period will be provided after the test.

Because you will be running downhill during the main trial, this is considerably easier than running on the flat or up hill. However, downhill running is likely to cause muscle soreness and weakness that will last up to one week after exercise; these responses are quite normal. This protocol has been used extensively in previous research investigating recovery from exercise-induced muscle damage.

During both exercises, you are free to stop at any point, we will show you how to do this during the first graded exercise test. In the event of injury, the researchers are first aid qualified and will provide acute first aid if necessary. In the event of cardiac arrest, there is an automated external defibrillator (AED) in the laboratory.

Blood sampling:
Blood will be drawn from a vein in your arm by a trained phlebotomist using standard procedures. Insertion of the needle may cause a short, sharp painful sensation. Bruising at the site of insertion may occur. The phlebotomist will ask if you have a tendency to feel faint when having blood sampled and whether you have an allergy to latex or the adhesive on medical tape. To ensure your comfort, we will ask you to sit or lie on an adjustable bed during blood sampling.

WHAT ARE THE POSSIBLE BENEFITS OF THIS STUDY?

You will get a measure of your maximum oxygen uptake, the most accurate measure of cardiovascular fitness.

The findings of this study may provide evidence for the use of different types of protein supplements to aid recovery from strenuous, muscle damaging exercise. Scientific evidence would provide piece of mind for consumers of this product and may increase its use by those wanting to improve recovery after sport and exercise.

WILL ANY COSTS BE REIMBURSED?

There is no cost to you in taking part in this study, except your time. Recruited volunteers will receive a $160 petrol voucher at the completion of the final visit.

WHAT IF SOMETHING GOES WRONG?

If you were injured in this study, you would be eligible to apply for compensation from ACC just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

WHAT WILL HAPPEN TO MY INFORMATION?

During this study the researchers will record information about you and your study participation. This includes the results of any study assessments.

Identifiable Information

Identifiable information is any data that could identify you (e.g. your name, date of birth, or address). Only researchers will have access to your identifiable information. However, if a study test gives an unexpected result that could be important for your health we will provide you with a letter to take to your usual doctor.

De-identified (Coded) Information

To make sure your personal information is kept confidential, information that identifies you will not be included in any report generated by the researcher. Instead, you will be identified by a code. The researcher will keep a list linking your code with your name, so that you can be linked to your coded data if needed. Only the researcher will have access to this list and be aware of the relationship between the coded information and the participant it belongs to.

The results of the study may be published or presented, but not in a form that would reasonably be expected to identify you.

Security and Storage of Your Information.

Your identifiable information will be held, under lock and key, at Practical Teaching Complex, Office 1.31 during the study. After the study it will be transferred to a secure archiving site and stored for at least 10 years, then destroyed. Your coded, de-identified information will be entered into electronic spreadsheets and kept by the researcher in secure, cloud-based storage indefinitely. All storage will comply with local and/or international data security guidelines.

Rights to Access Your Information.

You have the right to request access to your information held by the research team. You also have the right to request that any information you disagree with is corrected.

WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

The researcher will give you an overview of the main findings of the study and your personal results and answer any questions you may have about this research upon request. A significant delay may occur between data collection and the publication of any results, which is normal.

All study data will be stored for a maximum of 10 years, and will be the responsibility of the Principal Investigator, after which all files will be destroyed.

All data collected in this study will not be used for future related studies or unrelated research.

Blood collected from you in this study will not be passed on to anybody else outside of this project and will be destroyed after being analysed.

If you decide to withdraw from the study, your health questionnaire can be returned to you upon request, otherwise it will be destroyed. Any blood samples collected from you will be destroyed. All data collected from you prior to your withdrawal from this study will not be included in the analysis and overall findings from this project unless you give us permission to include it.

CAN I FIND OUT THE RESULTS OF THE STUDY?

You will be provided with a summary of the results once analysis has been completed. The results of this study, including raw de-identified data sets, will be published in an international, peer reviewed scientific journal and may be presented at scientific conferences. Additionally, this study is registered with Australian and New Zealand Clinical Trials Register, details of the study can be found on their website under ACTRN12622000529741p.

WHO IS FUNDING THE STUDY?

This project is co-funded by the Ministry of Business, Innovation and Employment through the High Value Nutrition National Science Challenge and Southern Pastures Investments and Ovation Ltd.

WHO HAS APPROVED THE STUDY?

This study has been approved by an independent group of people called a Health and Disability Ethics Committee (HDEC), who check that research studies meet established ethical standards. The Central Health and Disabilities Ethics Committee has approved this study.

WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Researcher: *Dr Matthew Barnes*
Phone: *06 9516822*
Email: *m.barnes@massey.ac.nz*

If you want to talk to someone about Māori cultural support, you can contact:

Dr Bevan Erueti
Associate Dean Māori, College of Health, Massey University
Phone: *06 9516087*
Email: *B.Erueti@massey.ac.nz*

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050
Fax: 0800 2 SUPPORT (0800 2787 7678)
Email: advocacy@advocacy.org.nz

Website: https://www.advocacy.org.nz/

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone: 0800 4 ETHIC
Email: hdecs@health.govt.nz

Consent Form

Effect of collagen hydrolysate on recovery from downhill running

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Please tick to indicate you consent to the following

I have read and I understand the information sheet for volunteers taking part in this study.	
I have had sufficient time to discuss this study and I am satisfied with the answers I have been given.	
I have had the opportunity to use a legal representative, family, whanau support or a friend to help me ask questions and understand the study.	
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without giving a reason.	
I understand that my participation in this study is confidential and that no material, which could identify me will be used in any reports on this study.	
I understand that my health questionnaire will only be assessed against the inclusion and exclusion criteria of the study. Only the principal investigators will see or have access to this information. No other person will see my questionnaire.	
I understand that ACC-equivalent compensation provisions will be provided to me in case of injury or illness as a result of participating in this study.	
I know who to contact if I have any questions about the study in general.	
I understand my responsibilities as a study participant.	
In the event of any significant abnormal results being obtained during the study, I agree that I will discuss these with my GP.	Yes / No
I wish to receive a summary of the results from this study.	Yes / No
	Yes / No

Yes / No

Declaration by participant:
I hereby consent to take part in this study.

Participant's name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature: Date:

Appendix C: Health Screening Form

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

HEALTH SCREENING FORM

VERSION 1 (11/10/2021)

Personal details

Name: _____

Age: _____

Contact details: _____

_____

Emergency contact

Name: _____

Contact details: _____

_____

Family Doctor

Name: _____

Contact details: _____

_____

Do not complete - to be completed during screening by the researcher

Height_____

Weight_____

Health history

Have you or anyone in your family ever experienced any of the following? (tick for yes)

- ☐ High blood pressure
- ☐ Low blood pressure
- ☐ Heart problems
- ☐ Stroke
- ☐ Breathing problems
- ☐ Lung fibrosis disorders
- ☐ COPD/chronic obstructive pulmonary disease
- ☐ Cancer or tumours
- ☐ Asthma
- ☐ Diabetes
- ☐ Epilepsy
- ☐ Arthritis
- ☐ Kidney/bladder disorders
- ☐ Stomach disorders
- ☐ Hernia
- ☐ Allergies
- ☐ Chronic conditions (e.g. lupus)
- ☐ Other (please identify) _____

If yes to any of the listed conditions, please explain:

_____

_____

_____

_____

Have you recently (in the last month) had any bacterial or viral related illness (if yes, please give details)?

Yes *No*

_____

_____

_____

Have ever had any surgery or trauma to your nose?	Yes	No
Have you ever been told that you snore loudly?	Yes	No
Have you ever been told that you stop breathing during your sleep?	Yes	No
Do you have a deviated septum?	Yes	No
Do you have difficulty breathing through your nose?	Yes	No

Is there any information, not discussed, that you feel is relevant?

I (print name) _____ have given true and complete information to the best of my knowledge.

Signature: _____ Date: _____

Researcher: _____ Date: _____

Appendix D: 24-hour Diet Record

MASSEY UNIVERSITY
COLLEGE OF SCIENCES

HUMAN NUTRITION RESEARCH UNIT
SCHOOL OF FOOD & ADVANCED TECHNOLOGY, COLLEGE OF SCIENCES

24h Diet Record

A 24-hour record involves research participants recording and describing all the food and drink consumed in the previous 24 hours, from waking to sleeping.

The record should include all food and drink consumed during the 24hr period including all meals, snacks, drinks, "nibbles", sweets, nutrient supplements etc. You can use headings such as 'early morning', 'breakfast', mid morning', 'mid day', 'mid afternoon', 'evening meal', 'late evening' and 'bedtime'.

If possible, record the food close to the time you consumed it. Do not worry about your writing – as long as we can read it, it is fine. We are not worried about pen colour, corrections, or the form getting stained in the kitchen.

The more accurate you are in recording the food you eat, the more accurate your nutritional analysis will be.

Instructions:

1. **Please write down everything you eat and drink for one full day** (from the time you wake up until the time you go to sleep). Try not to change your diet in any way. This day should represent your normal eating pattern. Do not pick an atypical day, such as a day you have a party, a holiday, weddings, etc. Include the approximate time of eating or drinking

2. **Be as accurate as possible when recording amounts**. Try to record your food intake as soon as you eat, otherwise it can be hard to remember at the end of the day. If you have mixed foods or casseroles, write down all the ingredients and amounts (salad: type of lettuce, croutons, vegetables, cheese, meat, dressing). Write down if foods are fresh, frozen, or canned and the brand name (eg Pams). For example, include specific detail such as ½ cup low-fat ("light blue") milk, 1 slice toast bread, 8 oz. fat-free fruit Yoplait yogurt, 200mL calcium fortified orange juice.

Tips:

a) Enter only one food item per line
b) Use measuring spoons (or weigh) for items such as jam and condiments.
c) Use measuring cups (or weigh) for items such as vegetables, pasta, rice, cereals if you have these available
d) Use g or dimensions (or weigh) for meat, cheese, pizza, desserts – you can compare eg with a pack of playing cards.
e) Use number and size (small, medium and large) for bread rolls, raw fruits, etc.
f) Use mL or cups for beverages.
g) Record the date, time and place (home or restaurant) of every meal and snack, as well as the method of preparation (fried, baked, barbecued, grilled, etc.).
h) Feel free to provide recipes or labels of unusual foods (photos are wonderful).
i) Please list dietary supplements (vitamins, minerals, protein powders etc.) – labels are really useful here! The more information the better.
j) If you drink from a water bottle you can just record how much is drunk throughout the day, rather than every time you take a drink.

Example

Time/meal	Food/drink - description	Amount	
Early morning	Mug of coffee, filter-type, Jeds No4	200 mL	
	Light blue milk	40mL	
	Digestive biscuit, Griffins	1	
Breakfast ~7.30	Porridge, Pams instant oats	40 g	
	Light blue milk	5 tbsp	
	Honey, Pams	1 dsp	
Midmorning	Snickers bar (1 mini bar from multipack)	1	
	Orange juice from Massey student cafe	Glass - about 200mL	
Lunch	etc		
Throughout day	Tap Water from own bottle (size 621m mL)	3 and a half bottles	

24h Diet Record Date: Typical day? Y/N

Time/meal	Food/drink - description	Amount	

Appendix E: VAS

Muscle Soreness Questionnaire

Subject Name:

Date/time:

0 **10**

0 = No soreness

10 = Very, very sore

Appendix F: Raw Data for Blood Samples

RAW CK	PRO					PLA					CH				
1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
108	139	323	201	128	101	137	322	198	141	198	243	1004	517	316
181	215	476	237	145	154	180	382	240	212	626	726	1295	933	668
110	156	377			5616	2992	1794	839	763	445	486	1076	527	354
2298	2601	3324	1421	823	218	282	699	399	227	85	117	349	162	124
122	153	245	94	86	51	110	250	117	72	171	219	562	237	140
278	319	556	332	346	459	495	341	230	163	509	644	1118	995	614
225	319	956	537	314	41	120	530	298	216	150	205	557	323	249
689	930	1331	766	622	167	191	496	290	175	298	1292	908	639	297.5
500	446	1066	502	357	1198	1381	1111	571	419	3120	4205	3773	1907	893
380	468	1125	1264	761						293	340	857	603	367
277	348	530	325	202										
469.82	554.00	937.18	567.90	378.40	889.44	654.22	658.33	353.56	265.33	589.50	847.70	1149.90	684.30	402.25
632.29	714.83	871.67	452.18	267.26	1808.97	964.75	499.50	223.73	209.02	905.61	1230.25	966.80	507.52	246.75

RAW HCRP	PRO					PLA					CH				
2.18	2.15	2.2	1.68	1.24	1.07	1.04	2.51	4.21	3.04	0.2	0.19	0.69	0.48	0.3
0.49	0.47	0.42	0.38	0.37	0.32	0.32	0.96	0.89	0.75	0.44	0.45	0.58	0.43	0.32
0.72	0.7	0.9			0.18	0.17	0.18	0.15	0.13	1.07	0.94	1.45	1.06	1.05
0.14	0.15	0.31	0.21	0.19	0.29	0.3	0.29	0.21	0.16	1.78	1.77	3.02	2.04	1.48
1.58	1.67	1.14	0.74	0.53	0.8	0.74	1.21	0.86	0.79	0.51	0.48	7.4	4.29	2.47
0.3	0.29	0.27	0.2	0.18	1.36	1.33	2.28	2.11	1.45	0.08	0.08	0.17	0.17	0.14
0.23	0.2	0.4	0.26	0.23	1.23	1.23	2.46	2.03	1.44	0.47	0.47	0.43	0.31	0.29
0.36	0.36	0.58	0.49	0.43	0.37	0.34	0.85	3.83	8.92	0.17	0.18	2.58	17.24	27.86
0.58	0.63	0.98	0.64	0.51	5.17	4.97	4.73	3.54	2.73	2.33	2.33	1.65	1.13	1.74
0.28	0.29	0.33	1.04	0.63						2.13	2.48	3.07	2.11	1.78
1.21	1.23	1.15	0.83	0.7										
0.73	0.74	0.79	0.65	0.50	1.20	1.16	1.72	1.98	2.16	0.92	0.94	2.10	2.93	3.74
0.65	0.66	0.58	0.46	0.32	1.55	1.49	1.44	1.57	2.73	0.86	0.92	2.15	5.18	8.51