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THE EFFECTS OF HYDROLYZED COLLAGEN ON PERFORMANCE, SORENESS AND COLLAGEN BIOMARKERS FOLLOWING EXERCISE IN RESISTANCE TRAINED MALES

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THE EFFECTS OF HYDROLYZED COLLAGEN ON PERFORMANCE, SORENESS AND COLLAGEN BIOMARKERS FOLLOWING EXERCISE IN RESISTANCE TRAINED MALES

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Abstract

Hydrolyzed Collagen (HCOL) is a nutritional supplement that has recently been suggested to enhance musculoskeletal (MSK) resilience and recovery from damaging exercise. Despite some promising evidence in clinical and *in vitro* models, there is a paucity of data in regards to its efficacy in healthy, exercising humans. **PURPOSE:** The purpose of this study was to determine whether consuming a HCOL supplement could be beneficial for well-trained individuals by examining the effect on acute recovery from high-force eccentric exercise. **METHODS:** Resistance trained males consumed 15g per day of either HCOL or a cornstarch placebo (PLA) for 12-days. After 7-days, they had a number of outcome variables measured, including: maximal voluntary isometric contraction (MVIC), countermovement jump performance (CMJ), lower body soreness, pain pressure threshold (PPT) tests and collagen turnover biomarkers (PICP and ICTP). After collection of baseline measures, participants performed a muscle damage protocol (5 sets of 20 drop jumps from 60cm box) before having every variable measured again at the following time points: immediately post (IP), 24h post (24h), 48h post (48h) and 120h post (120h). **RESULTS:** In both groups, lower body muscle soreness assessed via VAS was significantly higher than PRE at 24h ($p=0.001$) and 48h ($p=0.018$), but had returned to baseline by 120h ($p>0.05$). MVIC in both legs showed a significant time effect (Left: $p=0.007$; Right: $p=0.010$) over the 5-day post damage period. These finding indicate that the participants were sufficiently damaged using the drop jump protocol. Compared to baseline (HCOL = 38.2±9.2cm vs. PLA = 38.7±7.8cm), CMJ height performance was maintained in the HCOL group at 24h, whereas the PLA group experienced a significant decline (24h: HCOL = 37.0±8.4cm vs. PLA = 33.7±7.6cm; $p<0.001$). Neither the collagen synthesis (PICP) or collagen breakdown (ICTP) biomarkers changed significantly.

CONCLUSION: In summary, at the 24 h time point, HCOL attenuated the performance decline that is typically associated with muscle damage. This finding suggests that short term consumption of HCOL either enhanced tissue repair/recovery rate or reduced the magnitude of damage incurred by the drop jump protocol.

Key Words: Hydrolyzed Collagen, Recovery, Exercise Induced Muscle Damage, Connective Tissue

Chapter 1 – Introduction

Following an acute bout of intense exercise, it is common to incur some degree of musculoskeletal (MSK) tissue damage. It is well known that exercise induced muscle damage (EIMD) can transiently inhibit performance by disrupting ultrastructural myofibres, leading to a reduction in force production capacity, an increase in muscle soreness and an efflux of myocellular proteins into the circulation (Peake et al., 2016; Twist & Highton, 2013; Clarkson & Hubal, 2002). Whilst a certain degree of metabolic and mechanical stress is required to initiate tissue remodeling and repair (Hyldahl & Hubal, 2014), accelerating the recovery from this damage may be favorable for improving consecutive athletic performances. In addition to the well-researched effects on muscle tissue, there is evidence that eccentric exercise also damages connective tissues (Brown et al.,1997), including the extracellular matrix (ECM) which surrounds muscle fibers (Mackey et al., 2004). Traditionally thought to act as a simple scaffold network, recent technological advances have shown that the ECM is not only responsible for biological mechanotransduction, but that it is a complex dynamic tissue involved in signaling and regulatory processes within muscle (Gillies & Lieber, 2011). Recent data has demonstrated an association between ECM remodeling and the repeated bout effect (RBE) phenomenon (a protective adaptation in which an individual exposed to an initial bout of damaging exercise, will incur substantially less tissue damage during a subsequent bout of similar exercise) (Hyldahl et al., 2015). If the remodeling process of the ECM can be accelerated or improved, there is the possibility that a direct performance benefit could manifest due to enhanced mechanotransduction and passive force generation. Another intriguing possibility is whether augmenting the recovery capacity or resilience of the ECM could result in better protection

against subsequent tissue damage (by enhancing the effectiveness of the RBE), and thus reducing transient performance decrements as well as reducing the risk of MSK injury. Indeed, several hormone and drug treatments appear to be able to modulate this connective tissue adaptation (Mackey & Kjaer, 2016), however it is not yet clear if this process can be enhanced through more readily available interventions, such as nutritional manipulation or supplementation.

From a practical perspective, recovery has been defined as the ability to meet or exceed previous performance in a particular activity (Bishop et al., 2008). Optimizing recovery rate from muscle damage or injury is important for consecutive athletic performances as well as for accumulating training volume and thus adaptation (Raysmith & Drew, 2016). Upon return to a resting state, the body will initiate metabolic (restoration of fuel levels) and mechanical recovery processes (reparation of musculoskeletal (MSK) system damage) (Heaton et al., 2017), and both nutrition and rest can significantly influence these processes (Heaton et al., 2017). Nutritional strategies used to enhance acute recovery may target a variety of areas, such as the restoration of muscle glycogen (Beelen et al., 2010), reparation of muscle tissue (Phillips & Van Loon, 2011), reduction of inflammation (Urso & Sawka, 2013) and/or support of the immune system (Gleeson et al., 2004). Furthermore, it has been suggested that nutritional supplements could modulate acute recovery following EIMD. Specific examples include protein/amino acids, dietary polyphenols and vitamins D, C & E (Owens et al., 2019), and results have generally been mixed in regards to their efficacy.

In addition to the direct performance decrements that can occur following insufficient recovery, when chronic training/performing is not properly matched with recovery, underperformance syndrome (UPS; also known as overtraining syndrome) can develop (Kreher, 2016). Underperformance syndrome can result in dysregulated performance for months and can

significantly increase the risk of an MSK injury developing (Soligard et al., 2016). MSK injuries make up a significant percentage of injuries to athletic populations, with ligamentous sprains and muscular strains accounting for approximately 46-60% of all acute injuries (Kerr et al., 2010). Chronic injuries such as tendinopathies are usually overuse-related and constitute around 30% of all resistance training injuries (Raske & Norlin, 2002). Becoming injured leads to time away from training to heal the tissue itself, as well as concomitant detraining effects of various organs and bodily systems (Mujika & Padilla, 2000). Developing strategies to reduce the occurrence of, or ameliorate the symptoms of these adverse events should be prioritized.

Within the human body, collagen is the most abundant protein and plays an integral role in the structure and function of many different tissues. Type I collagen predominately makes up ligament, tendon, fascia and the organic matrix of bone tissue. The Type 1 collagen in tendon and fascia provides tensile stiffness, and within bone it defines various biomechanical properties (Gelse et al., 2003). Additionally, collagen makes up a significant proportion of the aforementioned ECM (Velleman, 1999). Considering the ubiquity of collagens throughout the MSK system, ensuring that the body has sufficient quantities of its precursor amino acids has been hypothesized to provide a physiological benefit by supporting connective tissue reparation (Baar, 2015). Individuals who are regularly incurring damage to their MSK system may require greater quantities of these amino acids in order to sufficiently support growth and repair of skeletal muscle ECM, tendon, cartilage and bone tissue.

Hydrolyzed Collagen (HCOL) is a nutritional supplement suggested to be beneficial for enhancing MSK and connective tissue repair/resilience (Heaton et al., 2017). The proposed mechanism is that an increased concentration of collagen precursor amino acids may support a proliferation in collagen synthesis within target tissues (Baar, 2015). Results from animal models

have shown that HCOL can stimulate production of Type II collagen in the ECM of cartilage tissue (Oesser et al., 1999), as well as improve resistance of the Achilles tendon to rupture by promoting more rapid tissue remodeling (Vieira et al., 2014). A recent *in vitro* model utilizing human ligamentous tissue has indicated that HCOL supplementation can enhance collagen synthesis in response to gelatin (precursor to HCOL) supplementation within human connective tissue (Shaw et al., 2017). Engineered ligaments were found to have greater tensile strength and increased collagen content following 3 days of gelatin consumption, which has implications for injury prevention and recovery. Most recently, Clifford et al. (2019) determined that supplemental HCOL benefitted the recovery of countermovement jump (CMJ) performance and muscle soreness following a damaging drop jump exercise protocol.

At present, there is a paucity of data in terms of the practical application and efficacy of HCOL supplementation in healthy populations. Additionally, there are reports of Sport Nutrition professionals recommending HCOL supplementation for athletes suffering from tendon or ligament injuries (Alcock et al., 2018). Further investigations are required before these supplementation recommendations can be considered effective. There is currently little to no research assessing whether 1) HCOL supplementation might improve acute recovery from exercise, 2) HCOL supplementation can decrease injury incidence or accelerate healing rate of injured MSK tissues, and/or 3) HCOL might be able to influence chronic adaptations associated with training. Also of interest would be the elucidation of the optimal dose and frequency of HCOL supplementation required to maximize efficacy.

The purpose of this study was to take the first step into determining whether consuming a HCOL supplement may be beneficial for well-trained individuals. A double-blind randomized control trial was performed, with one group consuming HCOL and the other group consuming an

inert placebo (PLA). Comparing changes between groups in markers of acute recovery and indices of connective tissue remodeling following a bout of high-force eccentric exercise will mark the initial attempt to determine whether consuming HCOL can provide a benefit within a highly resistance trained population.

Research Questions

1. Does taking a Hydrolyzed Collagen supplement improve acute recovery from a bout of high-force eccentric exercise in highly resistance trained individuals?
    - **1.1.** Do soreness levels return to baseline more rapidly with 12-days of HCOL supplementation compared to a placebo?
    - **1.2.** Does strength return to baseline more rapidly with 12-days of HCOL supplementation compared to a placebo?
    - **1.3.** Does jump performance (CMJ) return to baseline more rapidly with 12-days of HCOL supplementation compared to a placebo?
2. Does taking a Hydrolyzed Collagen supplement for 12-days provide a protective effect to the MSK system (i.e. reduced magnitude of performance decrements/physiological dysregulation) following a bout of high-force eccentric exercise in highly resistance trained individuals?
    - **2.1.** Is soreness lower following 7-days of HCOL supplementation compared to a placebo?
    - **2.2.** Does muscle function (MVC) decrease by a lower magnitude following 7-days of HCOL supplementation compared to a placebo?
    - **2.3.** Does jump performance (CMJ) decrease by a lower magnitude following 7-days of HCOL supplementation compared to a placebo?

3. Are serum markers of connective tissue breakdown (carboxy-terminal telopeptide of type I collagen, ICTP) or synthesis (carboxy-terminal propeptide of type I procollagen, PICP) affected by 12-days of HCOL supplementation or high-force eccentric exercise?

3.1. Are serum markers of connective tissue breakdown (ICTP) or synthesis (PICP) affected by the muscle damage protocol?

3.2. If serum biomarkers (ICTP or PICP) are affected by the muscle damage protocol, does 12-days of HCOL supplementation modulate this effect?

Alternate Hypotheses

1. Hydrolyzed Collagen supplementation will allow participants to recover from acute bouts of EIMD more rapidly due to faster repair of connective tissues (i.e. endomysium, tendons) associated with transferring forces within the muscle

1.1. There will be a faster return to baseline levels of soreness following an eccentric bout of exercise in the HCOL group compared to placebo.

1.2. There will be a faster return to baseline of muscle function (indicating restoration of force producing capacity) at the 24h and 48h time points in the HCOL group compared to the placebo.

1.3. There will be a faster return to baseline of jump performance (indicating restoration of force producing capacity) at the 24h and 48h time points in the HCOL group compared to the placebo.

2. 7-days of Hydrolyzed Collagen supplementation will provide a protective effect to the MSK system, resulting in a decreased physiological dysregulation of outcome measures

2.1. Soreness will be lower in the HCOL vs the PLA groups in the 24h and 48h time period following eccentric exercise

2.2. Muscle function will decrease by less in the HCOL vs the PLA group in the 24h and 48h period following eccentric exercise

2.3. Jump performance will decrease by less in the HCOL vs the PLA group in the 24h and 48h time period following eccentric exercise

3. Serum markers of connective tissue synthesis and breakdown will be significantly different at either the 24h, 48h, or 120h time period following the muscle damage protocol

3.1. Serum markers of collagen synthesis and breakdown will be elevated compared to baseline at either the 24h, 48h, or 120h time points

3.2. HCOL supplementation will enhance the increase of collagen synthesis biomarkers and cause a decrease in collagen breakdown biomarkers following the muscle damage protocol

Significance of the Study

Optimizing recovery is important for consecutive athletic performances. If HCOL supplementation can be shown to enhance recovery following an acute bout of damaging exercise, it may provide performance benefits to consecutive exercise bouts or afford protection against the development of UPS, leading to a reduction in the incidence of MSK injury.

Recent data suggests that connective tissue is more adaptive to exercise stress than previously thought. Given this, if HCOL supplementation is shown to be effective at enhancing the resiliency of the ECM, it may work synergistically with underlying mechanisms of the RBE to provide higher levels of protection during exercise and/or competition. Furthermore, ensuring the robustness of the skeletal muscle scaffold (i.e. ECM) may improve force transmission and passive force generation and thus provide a direct benefit to total force output and physical performance.

Since ingestion of supplemental collagen has previously been shown to increase collagen synthesis within human tissue, a better understanding of how this increased synthesis may physiologically promote recovery in resistance trained individuals following intense exercise is warranted. We believe that this is an important first step towards determining whether this supplement should be recommended to athletes.

Delimitations

- Participants will be males aged between 18-35 years of age
- Participants will be recruited from the University of Oklahoma and greater Cleveland county area
- Participants will be highly resistance trained: resistance training at least 3 days per week for the last 2 years (including lower body training at least once per week)
- Participants will be injury free and will not have suffered any significant lower body injuries 1 year prior to the study start date (unless they have been cleared to return to full activity/high intensity exercise by their physician for at least the same time period)
- Participants will not be currently taking any collagen supplementation (or have taken any in the 6 months prior to the start of the study period)

Limitations

- Participants will be young, resistance trained males and so observations/conclusions will be restricted to this population.
- Participants will not be sequestered and thus must be relied upon to take supplement daily whilst in free living conditions.

- Paucity of data in literature means it is unclear whether the duration of the study will be long enough for the supplements to exert a significant effect on the connective tissue and/or skeletal muscle.
- Concurrent exercise activity and diet during the supplementation period will not be controlled beyond asking the participants to maintain their usual diet and activity levels
- Markers of muscle and connective tissue damage will not be directly assessed

Assumptions

- Participants have not lied about training status/previous resistance training experience
- Participants will maintain usual dietary/supplement and daily routines throughout duration of the study
- Participants will follow all directions, including taking the prescribed supplement daily at the same time and in the same manner.
- Participants will provide a maximal effort during performance measure testing and the muscle damaging protocol
- Sufficient muscle and/or connective tissue damage will be incurred using this exercise protocol
- Biomarkers are sufficient proxies for muscle and/or connective tissue damage

Operational Definitions

1. **Hydrolyzed Collagen (HCOL) -** Collagen is extracted from the connective tissue of animal sources before being subjected to processes' including enzymatic hydrolysis, purification, and sterilization. Can be further hydrolyzed into gelatin, and then into a Hydrolyzed Collagen (also known as collagen peptides) powder for human consumption

2. **Extracellular matrix (ECM)** – collection of extracellular molecules that biochemically and structurally support surrounding cells. Ensures a functional link between skeletal muscle and bone. Includes the interstitial matrix and basement membrane (Kjaer, 2004)
3. **Exercise Induced Muscle Damage (EIMD)** – cellular damage to muscle fibers usually caused by eccentric exercise. Characterized by reduced force production, soreness and inflammation (Clarkson & Hubal, 2002)
4. **Underperformance syndrome (UPS** - condition of maladapted physiology caused by excessive volume or intensity of exercise without adequate rest/recovery. Can result in performance decrements and mood disturbances lasting months (Kreher, 2016)
5. **Carboxyterminal Propeptide of Type I Collagen (PICP)** – biomarker that reflects type I collagen synthesis (Langberg et al., 2000)
6. **Carboxyterminal Telopeptide Region of Type I Collagen (ICTP)** – biomarker that reflects type I collagen degradation (Langberg et al., 2000)
7. **Countermovement Jump (CMJ) –** jumping technique that involves an individual squatting down to approximately a 90° knee bend and then immediately jumping vertically as high as possible (can be performed with either hands on the hips or using an arm swing) (Hori et al., 2009)
8. **Maximal Voluntary Isometric Contraction (MVIC)** – standardized, objective assessment of maximal isometric muscle strength (Meldrum et al., 2003)
9. **Visual Analog Scale (VAS) -** A scale from 0-100 allowing individuals to subjectively estimate the degree of soreness experienced. A score of 0 indicates “no soreness at all” and 100 indicates the “most intense pain they can imagine” (Heller et al., 2016)

Chapter 2 – Literature Review

Millions of individuals around the globe regularly participate in exercise to enhance their health, well-being or athletic ability. To continue developing beneficial physiological adaptations, the magnitude of stress placed upon the body must be gradually increased (termed progressive overload), and thus greater volumes and/or intensities of exercise are required (Kraemer et al., 2002). High intensity exercise, especially involving a significant eccentric component can cause tissue damage which may inhibit subsequent exercise performance over the following days (Byrne et al., 2001). To optimize physical performance, it is important to maximize recovery from these acute bouts of intense exercise. Upon return to a resting state, the body will immediately begin both mechanical and metabolic recovery processes (Heaton et al., 2017). If recovery can be accelerated and injury avoided, individuals can train harder, longer and more frequently. Accumulating training volume is directly related to adaptation (Raysmith & Drew, 2016) and can result in better competitive or physical performance over time. Nutrition and rest have a substantial impact on recovery rate (Heaton et al., 2017), and so strategies to enhance these processes are of interest to researchers.

In addition to recovering faster from an acute training bout, avoiding musculoskeletal injuries is a high priority for regular exercisers. Intense exercise can result in acute, as well as chronic injuries. Raysmith and Drew (2016) found that athletes able to complete greater than 80% of their planned training weeks (i.e. not modified due to injury or illness) had a 7-fold greater likelihood of achieving their performance goals. If an athlete becomes injured, not only are they losing time away from training to heal the injury itself, but they may also experience detraining effects to their metabolic, muscular and cardiorespiratory systems (Mujika & Padilla, 2000). Additionally, they may now be more susceptible to suffering a repeat injury of the same

tissue, or a contralateral injury due to compensatory patterns (Rauh et al., 2007; Paterno et al., 2014). Therefore, the fewer injuries that an athlete can sustain over their career, the greater their athletic or physical potential could be.

Within the human body, collagen is the most abundant and plays an important role in the structure and function of different tissues. Hydrolyzed Collagen (HCOL) is a nutritional supplement that has recently become popular for individuals engaged in regular exercise. It has been theorized that providing the body with an adequate supply of collagen specific amino acids may enhance recovery or resilience of collagenous tissues within the body (Baar, 2015). Whilst there is a good amount of literature exploring the effectiveness of HCOL supplementation in clinical populations, there is very little data concerning its efficacy in healthy, exercising populations.

Therefore, the purpose of this literature review is to explore factors related to MSK injury and recovery, discuss current nutritional recommendations purported to improve acute recovery, evaluate the role of collagen within the human body and finally determine current gaps in the literature in regards to HCOL supplementation.

Exercise Related Injury Epidemiology

An injury can develop acutely (rapid onset secondary to traumatic event) or chronically (repetitive stress placed on a tissue that has insufficient recuperative ability) (Lavallee & Balam, 2010). In terms of MSK injuries, ligamentous sprains and muscular strains account for approximately 46-60% of all acute injuries (Kerr et al., 2010). Chronic injuries such as tendinopathies are usually overuse type injuries and make up around 30% of strength training related injuries (Raske & Norlin, 2002).

There are approximately 16.4 million cases of ligament and tendon injuries reported yearly in the US, with around 100,000 cases involving the Achilles tendon (Järvinen et al., 2005). Tanaka et al. (2001) evaluated reports of tendinitis in US workers throughout 1988, concluding that there were around 520, 000 total cases. Diabetics have a significantly higher risk of suffering a tendon injury compared to healthy populations (Abate et al., 2013) and it has been reported that that around 80% of individuals in their 80's who were assessed had experienced tendon damage (rotator cuff lesion) to some degree, implicating aging as a significant risk factor for connective tissue injury (Milgrom et al., 1995).

Both endurance and resistance exercise can result in injury. Data from distance runners has shown injury incidence rates ranging from 19.4 to 79.3% (van Gent et al., 2007). During routine training, 43.3% of powerlifters (0.3 injuries per lifter per year) and 45.1% of bodybuilders (0.12 injuries per bodybuilder per year) reported suffering from symptoms whilst exercising (Siewe et al., 2011; Siewe et al., 2014). A recent investigation into injury rates of CrossFit athletes determined the 6-month injury rate was approximately 20%, with the shoulders, knees and lower back becoming injured most frequently (Weisenthal et al., 2014).

In elite athletes, soft tissue injury incidence has been shown to be 60% in the Premier league for professional soccer players (Hawkins et al., 2001) and almost 70% during National Football League training camps (Feeley et al., 2008). Jacobsson et al. (2012) assessed data from Swedish elite track athletes and found that the 1-year prevalence of MSK injury lasting for more than 3 weeks was 43%, with approximately 90% of the recorded injuries affecting the lower extremities. More specifically, there is a high incidence of Achilles tendon injury that seems to have significantly increased over the last few decades. Some estimates have stated that Achilles tendon injuries may comprise as much as 30–50% of the total number of injuries related to sport

activities (Järvinen et al., 2005). Furthermore, the incidence of developing a tendinopathy in elite athletes at some point in their career has been shown to reach 45%, with symptoms (and potentially performance reduction) occasionally lasting for years (Lian et al., 2005). Athletes involved in high-volume, high-intensity training are susceptible to developing tissue damage (Close et al., 2019), which can lead to the development of overuse injuries if recovery is not sufficient (Lavallee & Balam, 2010).

Exercise Induced Muscle Damage

Following high force eccentric muscle contractions, a number of physiological responses can occur. These include increases in circulating intramuscular proteins, swelling of the affected limb, ultrastructural muscular disruption (fiber degradation and Z-line streaming), delayed onset of muscle soreness (DOMS), reduced range of motion and impaired capacity to produce muscular force (Owens et al., 2019). The foremost mechanism of primary muscle damage during eccentric exercise is considered to be mechanical loading. The lower motor unit recruitment associated with eccentric contractions results in a greater amount of stress being placed upon a smaller amount of fibers (Enoka, 1996). In addition, it has been observed that sarcomeres lengthen in a non-uniform way which can result in “sarcomere popping”, causing an increase in tension on passive structures and a subsequent deformation of non-contractile proteins within a muscle (Proske & Morgan, 2001). The secondary damage phase is characterized by an uncontrolled movement of calcium into the cell cytoplasm (Ebbeling & Clarkson, 1989) as well as an inflammatory cascade of immune cells moving in to stimulate apoptosis and clear damaged tissue (Chazaud, 2016). It is important to note that this immune response is necessary for tissues to recover and adapt in a normal fashion (Butterfield et al., 2006). Following a bout of eccentric exercise, it has been shown that muscles exhibit a reduced susceptibility to damage during the

next exercise bout. This phenomenon is known as the repeated bout effect (RBE), with proposed mechanisms including ECM remodeling, neural adaptations, alterations to mechanical properties and biochemical signaling (Hyldahl et al., 2017). To evaluate the magnitude of muscle damage incurred, a combination of force production capacity, soreness and serum concentrations of muscle-specific proteins (i.e. creatine kinase, myoglobin) must be assessed. Generally, the faster these variables return to baseline values, the better the recovery capacity of an individual. Several nutritional strategies have been suggested to enhance recovery following acute EIMD (Owens et al., 2019).

Nutritional Recovery Strategies

In recent decades, a substantial body of research has emerged with the goal of evaluating the efficacy of nutritional manipulation and supplementation on acute recovery from exercise. Whey protein has been recognized to augment acute recovery from damaging exercise in a variety of populations and scenarios (Buckley et al., 2010; Cooke et al., 2010; Brown et al., 2017). There is less clarity with other supplements such as amino acids (Ratamess et al., 2003; Howatson et al., 2012), tart cherry juice (Howatson et al., 2010) and curcumin (Nicol et al., 2015) due to a paucity of studies or contrasting findings. A recent review by Heaton et al. (2017) assessed different nutritional modalities for enhancing recovery in team sport athletes. Presently, there is "good" evidence to suggest that consuming sufficiently high protein, carbohydrate, fluids (both post workout and total daily) and creatine monohydrate can positively affect recovery rate in athletes. Supplements with "fair" evidence include omega-3 fatty acids, vitamin D, antioxidants and collagen + vitamin C.

Hydrolyzed collagen peptide supplementation (HCOL) is a relatively new area of study in terms of athletic recovery/injury prevention augmentation. A recent in-vitro model provided

initial evidence that HCOL supplementation can enhance collagen synthesis in response to loading, which has implications for injury prevention and recovery (Shaw et al., 2017). The recent study by Clifford et al. (2019) is the first to show a performance benefit for short term HCOL supplementation. Despite this, there is currently not enough data to categorically state whether HCOL is a useful supplement for young, healthy individuals to consume.

Collagen Overview

Collagen is the most abundant protein within the human body (approximately one third of total protein stores) and plays an integral role in the structure and function of an array of different tissues (Hashim et al., 2015). There are at least 28 different types of collagen, with type I being the most abundant in humans (Magnusson et al., 2016). Type I collagen predominately makes up ligament, tendon, myofibril endomysium and the organic matrix of bone tissue. Other collagens of note include Type II, III & IV collagen. Type II forms the articular and hyaline cartilage, Type III is incorporated into aortic and dermic tissue and Type IV makes up the basement membrane of cells (Bächinger et al., 2010). Collagen Types I through III are fibrillar in nature and contain a single triple-helical domain consisting of three different peptide chains which wrap around each other forming an alpha helix linked together by disulphide bonds (Velleman, 1999). The Type 1 collagen in tendon and fascia provides tensile stiffness, and within bone it defines biomechanical properties such as load bearing, tensile strength and torsional stiffness (particularly after calcification) (Gelse et al., 2003). Depending on fibril arrangement, tissues made up of collagen can exhibit anisotropic or nearly isotropic mechanical properties (Fratzl, 2008).

In addition to providing the biomechanical properties that allow functioning of the aforementioned tissues, collagen (Type I & III) makes up a significant proportion of the extracellular matrix (ECM) surrounding skeletal muscle (Velleman, 1999). The ECM acts as a biological mechanotransducer to prevent premature mechanical failure by storing and transmitting energy derived from deformation of muscle, as well as aiding in heat dissipation from muscular contractions (Silver & Landis, 2008). Figure 1 below provides an excellent visual representation of how the collagenous endomysium houses and supports muscle fibers (Trotter & Purslow, 1992). In recent years, the ECM has been shown to be more than a simple scaffold. Recent advances in technology have demonstrated that the ECM acts dynamically to regulate cell behavior (Welsh et al., 2014) via cell-ECM signal transduction pathways, ECM molecule interactions and growth factor interactions (Velleman, 1999). Furthermore, there are specific receptors that mediate the interaction with collagens, including integrins, discoidin-domain receptors, glycoprotein VI and specialized proteoglycan receptors (Vogel, 2001; Levine & Nishiyama, 1996). Signaling from these receptors can influence differentiation, adhesion, growth, cellular reactivities and cell survival (Gelse et al., 2003). In addition to this, collagens are involved in the entrapment, local storage and delivery of cytokines and growth factors (Yamaguchi et al., 1990) and thus may play a role in wound healing and tissue repair.

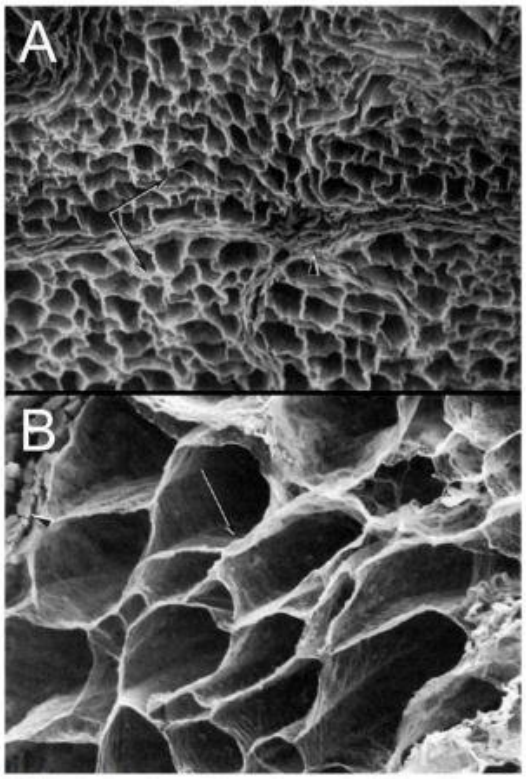


Figure 1. Scanning electron micrograph of the collagenous endomysial network around muscle fibers observed after digesting fibers with. This image suggests that muscle fibers are embedded in a complex connective tissue matrix and are intimately associated with ECM (Trotter & Purslow, 1992).

Response of Tissue Collagens to Exercise

There is evidence that performing exercise results not only in the breakdown of muscle tissue, but of connective tissue as well. Brown et al. (1997) found that urinary indices of collagen breakdown were elevated following a single bout of 50 eccentric contractions of the knee extensor musculature. In addition, Mackey et al. (2004) found that a single bout of high force eccentric contractions resulted in remodeling of the ECM; and more specifically a breakdown of endomysial type IV collagen (primary collagen found in the muscle basement membrane (Gillies & Lieber, 2011)) via the Matrix Metalloproteinase (MMP) pathway. Forces generated by the myofiber are transmitted to the ECM and then onwards to the tendon, which allows force transfer

to occur laterally over the perimeter of each myofiber (Grounds et al., 2005). As well as enabling this lateral force transmission, the ECM is important for structural integrity and the relay of mechanical signals within the muscle, in addition to regulating satellite cell self-renewal capability (Urciuolo et al., 2013). Eccentric exercise has been shown to cause greater damage to connective tissue compared to concentric exercise (Nogueira et al., 2011) and techniques such as downhill running (Welsh et al., 2014), eccentric isokinetic contractions (Brown et al., 1997) and plyometric exercise (Tofas et al., 2008) have all been shown to increase markers of collagen degradation. The time course of skeletal muscle collagen synthesis has been shown to mirror that of myofibrillar and sarcoplasmic proteins, expressing an elevation 6h post exercise, peaking 24h post exercise and remaining elevated at 48h post exercise (Miller et al., 2005). This finding supports the idea of coordinated musculotendinous adaptation.

To further support this concept, exercise can modulate connective tissue size, structure and function in response to training. Research has shown that endurance training is associated with an increased cross-sectional area of the Achilles tendon (Kongsgaard et al., 2005). Couppe et al. (2008) found that fencers and badminton players had a 20-30% larger patellar tendon in their lead leg vs their trail leg. Other studies have shown no alterations in tendon thickness following endurance (Hansen et al., 2003) or resistance exercise (Kubo et al., 2006), but instead an altered modulus which indicates that the composition of the tendon structure itself may be subject to change (Couppe et al., 2008). In addition to these findings, Farup et al. (2014a) determined that consuming whey protein whilst performing strength training for 12 weeks caused patellar tendon hypertrophy in young males. It has yet to be fully elucidated how exercise may affect connective tissue adaptations, however it is clear that connective tissues are dynamic and able to adapt to certain stimuli.

Considering the ubiquity of collagens within the MSK system, ensuring that the body has sufficient quantities of its precursor amino acids may be of benefit. The amino acids proline and glycine have been shown to be essential for the synthesis of new collagen (Li & Wu, 2018). Individuals who are regularly incurring greater damage and/or stress to their MSK system may require greater quantities of these amino acids in order to support growth and repair of the skeletal muscle ECM, tendons, cartilage and bone. Therefore, it would be of value to assess whether collagen supplementation could benefit athletes/well trained individuals.

Collagen Supplementation Overview

Collagen is extracted from the connective tissue of bovine, porcine or marine sources before being subjected to a number of processes that include: extraction, enzymatic hydrolysis, purification, concentration, sterilization, and drying (Wang et al., 2017). The core amino acids that compose its polypeptide chain are glycine, proline and hydroxyproline (Yamazaki et al., 2010), of which relative proportions may vary depending on the original animal source (Mahmood et al., 2016). The proteins that constitute collagen range in size from 0.5 to 13.5 kilodaltons (kDa), with a mean size of 3.3 kDa (Clark et al., 2008). Collagen is a non-gelling substance that dissolves in cold water. Gelatin is a compound derived from collagen, produced via partial hydrolysis of collagen (Figure 2 below). When dissolved in hot water, it will set to a gel upon cooling. Gelatin can be further hydrolyzed into collagen peptides (HCOL), which are sometimes referred to in the literature as: “collagen hydrolysate”, “hydrolyzed collagen”, “collagen peptides”, “gelatin hydrolysate” or “hydrolyzed gelatin”. Clinical trials have confirmed that collagen is a safe and generally well tolerated therapy (Zhang et al., 2008) and the Food and Drug Administration (FDA) center for food safety and nutrition recognize HCOL as a product safe for human consumption. Alcock et al. (2018) compared different food and supplements to

determine whether collagen specific amino acid concentration varied between sources. They found that there was a large degree of variability between sources, and that neither commercial nor self-prepared beef broth provided a reliable source of collagen specific amino acids. Interestingly, it seems that amino acid concentrations between commercial gelatin powder (Dr. Oetker, Mentone,VIC, Australia) and specifically formulated hydrolyzed collagen powder (Professional Whey, NSW, Australia; Tendeforte, Gelita, Eberbach, Germany) provided a similar quantity and proportion of amino acids per serving (Alcock et al., 2018).

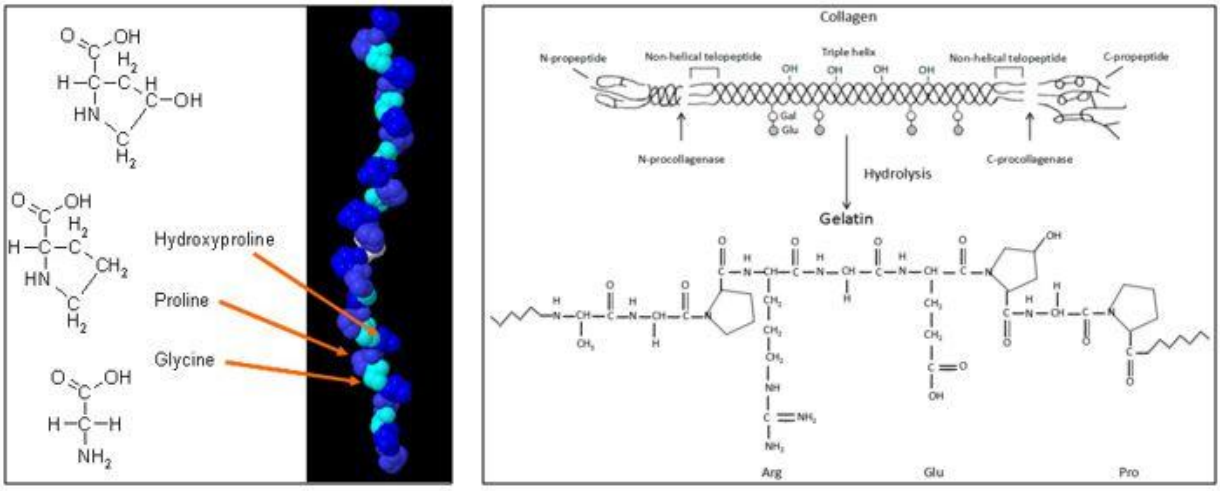


Figure 2. Molecular structure of collagen (Left) (Yama et al., 2017) and schematic description of conversion of collagen to gelatin (Right) (Yamazaki et al., 2010)

Initial preclinical trials showed that HCOL was able to pass across the mucosal barrier in the small intestine and remain as a polypeptide without being enzymatically cleaved into its constituent amino acids (Oesser et al., 1999). Following absorption, it has been shown to accumulate in the extracellular matrix of cartilage tissue and stimulate production of type II collagen (Oesser et al., 1999). Findings such as this led to research exploring the effects of HCOL supplementation within animal models, as well as within humans suffering from compromised articular cartilage (i.e. osteoarthritis (OA)).

Collagen Supplementation in Animal Models

Oesser, Raabe & Schunk (2007) assessed the effect of HCOL in mice bred to develop OA. After a 3-month period, they found a significant reduction in knee cartilage degeneration in the animals that had been fed HCOL. They concluded that HCOL may delay or even halt destruction of knee cartilage in mice with OA. The same research group then performed a study to determine the effect of HCOL in mice that had yet to develop OA, finding that prophylactic treatment with HCOL retarded the progression of OA by reducing cartilage degeneration in the knee (Oesser, Proksch & Schunck, 2008).

Glycine is one of the primary amino acids that makes up collagen and has been shown to perform a variety of different biological activities, such as modulating the systemic inflammatory cascade, improving microcirculation and assisting in the inhibition of Tumor Necrosis Factor α (TNF-α) and Interleukin-1β (IL-1β) (Hartog et al., 2007). Vieira et al. (2014) investigated the effect of a 5% glycine diet in rats as a treatment for the inflammation of the Achilles tendon. They discovered that after following the higher glycine diet, Achilles tendons were more resistant when being loaded until rupture point. They posited that glycine had induced synthesis of vital components of the tendon, promoting a more rapid remodeling of the tissue. The authors determined that glycine may be a beneficial supplement for humans with an inflamed Achilles tendon. However, until this result is replicated within a human population, caution must be taken before this conclusion can be reached.

Collagen Supplementation in Clinical Populations

Several clinical studies performed in OA populations have indicated that HCOL is a useful modality for reducing joint pain, decreasing dependency on analgesics, and improving leg strength (Zuckley et al., 2004). During a randomized, double-blind and placebo-controlled study,

Moskowitz (2000) determined that HCOL may have potential utility in the treatment of OA. A review by Bello & Oesser (2006) assessed data from four open-label and three double blind studies related to the effect of HCOL on subjects with OA. Kumar et al. (2015) concluded that collagen peptides may be a useful therapeutic intervention for managing OA and joint health. Despite the variability between studies, it seems that HCOL can provide symptomatic relief to individuals suffering from OA. In a more recent study, McAlindon et al. (2011) found that individuals suffering from mild knee OA increased their proteoglycan (proxy indicator of cartilage health) content following 24-weeks of HCOL supplementation. Furthermore, there is evidence that supplementation with Type II collagen can be a safe and effective modality for the treatment of rheumatoid arthritis (Zhang et al., 2008). It was proposed that the collagen may have caused a down-regulation of pro-inflammatory cytokines (IL-1 & TNF-α) and upregulation of suppressive cytokines (Transforming Growth Factor β & IL-4). In summary, administration of 7-10g of HCOL every day for 12 weeks has typically led to improvements in outcome measures within an OA population.

The following studies have assessed the effectiveness of HCOL supplementation for a number of different applications. Many of these studies have never been replicated or have been produced from the same research group, so should be viewed more critically until independently confirmed. Firstly, it has been reported that HCOL can accelerate the repair of damaged tissues, with Lee et al. (2006) reporting that individuals suffering from pressure ulcers had an almost doubled healing rate compared to the placebo group. Secondly, a recent study by König et al. (2018) provided HCOL to 66 postmenopausal women for 12 months to determine the effect on bone mineral density (BMD). They found significantly higher T-scores for the spine and femoral neck, as well as significantly higher levels of N-terminal peptide of pro-collagen I (PINP) (serum

biomarker of bone formation) in the experimental group, suggesting supplementation had a clinically beneficial effect. A study by the same group (Zdzieblik et al., 2015) assessed the effect of HCOL supplementation on strength and muscle mass in elderly sarcopenic males. This was the first study to investigate the effect of HCOL on body composition and muscular performance. They found that those supplementing with HCOL saw greater gains in fat free mass (FFM) and quadriceps muscle strength compared to controls. More recent evidence has shown that the collagen dipeptide Hydroxyprolyl-Glycine can induce myogenic differentiation and myotube hypertrophy via the PI3K/Akt/mTOR pathway (Kitakaze et al., 2016), so perhaps HCOL supplementation could influence FFM through this mechanism. However, caution must be taken when interpreting the results of this study due to the extraordinarily high concomitant increases in FFM and reductions in fat mass (FFM increased by 4.2kg; FM decreased by 5.4kg) achieved by an elderly population. As highlighted by the rebuttal submitted to the journal by Phillips et al. (2016), the validity of these results should be questioned.

Finally, HCOL has recently been reported to exert modulatory effects on the human circulatory system. Kouguchi et al. (2013) assessed the effect of HCOL supplementation on 58 individuals with either mild hypertension or high-normal blood pressure levels. They found that arterial stiffness (a marker of vascular damage) was significantly lower in the experimental group vs the control group. In addition, the blood pressure of the experimental group was significantly lower than that of the placebo group. They attributed these findings to a reduction in inflammatory markers within the microcirculation induced by the bioactive peptides Pro-Hyp and Hyp-Gly (originating from the HCOL). It has become apparent that collagens are involved in more sophisticated and subtle functions throughout the human body. Some data suggests it may

even stimulate angiogenesis and tumorigenesis which has attracted interest for pharmaceutical application (O'Reilly et al., 1997).

Collagen Supplementation in Athletes

With data available showing the effectiveness of supplementing with HCOL in animal models and clinical populations, interest has turned toward whether athletic individuals may derive a benefit. An observational study performed by Flechsenhar & Alf (2005) provided 100 elite German athletes with 10g/day of HCOL for 12 weeks and found that 78% of subjects reported reductions in joint pain or improved movement following the trial. The design of this study was a "postmarketing surveillance study" that did not incorporate a control group, so the implications of these results are unclear. A study by Clark et al. (2008) sought to replicate the findings of the German study in a more controlled manner (i.e. as an RCT). They provided 10g of HCOL to 147 student athletes who had previously complained of joint discomfort due to joint stress, injury, surgical outcome, or trauma. After a 24-week period, statistically significant reductions in pain were observed, with an even greater improvement when looking at a subgroup analysis of knee arthralgia. Recently, Gonçalves (2017) performed a case series evaluating the effect of HCOL on middle aged athletes with osteochondral lesions. MRI images showed restoration of articular surfaces following HCOL supplementation combined with physiotherapy in all 3 cases. Although the results seem positive, case studies have some significant methodological limitations, so these results must be viewed with caution. In addition, another recent study by Dressler et al. (2018) found an improvement in subjectively perceived ankle stability in athletes with chronic ankle instability, as well as a reduction in re-injury rate of ankle sprains after a 3 month follow up period. The findings of this study were attributed to enhanced ligament and tendon firmness due to increased ECM molecule expression induced by the

supplement. However, it must be noted that mechanical ankle stability as determined using an ankle arthrometer (to determine anterior talar drawer) did not show any difference between groups.

A recent study by Shaw et al. (2017) assessed the effect of vitamin C enriched gelatin supplementation combined with rope skipping on tendon collagen synthesis. Vitamin C was added because it functions as a cofactor during the tendon cross-linking process and is necessary for the hydroxylation of lysine and proline on procollagen. Individuals consumed either 5g or 15g of Vitamin C enriched gelatin or a placebo control before performing rope skipping 1 hour later for 3 days. Engineered ligaments showed increased collagen content and improved mechanics after being treated with serum from the gelatin supplementation group. In addition, PINP levels were doubled in those who consumed 15g of gelatin, indicating increased collagen synthesis within the body. This is the first study to demonstrate that a nutritional intervention can directly augment collagen synthesis of human ligamentous tissue following exercise. This has implications for injury prevention and tissue repair by making connective tissue more resilient to failure from loading. The limitations of this study are that the tendons used to test tensile strength mimic developing sinews as opposed to mature adult tissue. Developing ligaments have more cells and less matrix, their rate of collagen synthesis is significantly higher, they express more developmental collagen isoforms, and they are much weaker than adult sinews (Baar, 2017). Furthermore, the sinews were bathed in serum extracted from the individuals who consumed the supplement, allowing them to be fully exposed to the amino acids/growth factors. Within humans, it is unclear whether enough of the ingested HCOL can reach tendon/ligaments to exert a modulatory effect. Regardless, this study is the first to provide in vitro, mechanistic evidence for the beneficial effect of supplementing with collagen specific amino acids on injury

prevention/sinew resilience in human tissue. Lopez et al. (2015) showed promising preliminary data in regards to HCOL supplementation on acute exercise performance. After 6 weeks of HCOL supplementation, their untrained participants were better protected during a secondary exercise bout, indicating that the supplement may have augmented the RBE. Recently, Clifford et al. (2019) showed the first evidence that short term (7-day) HCOL supplementation was able to improve recovery from high-force eccentric exercise (125 drop jumps from 60cm box). They speculated that the augmented recovery of CMJ performance was related to either increased collagen synthesis in connective tissues surrounding the lower limb muscles or a modulation of the inflammatory response during early stage tissue remodeling. There remains a paucity of data in terms of the practical application/efficacy of HCOL supplementation in healthy and athletic populations. Questions that still need to be answered include: (1) What are the minimal and/or optimal doses and frequency of HCOL ingestion required? (2) Does supplementation decrease injury risk or accelerate the return to play after injury? (3) Can supplementation with HCOL improve performance? (Heaton et al., 2017).

Conclusion

In summary, HCOL supplementation has been shown to have potentially beneficial effects on tendon and ligament strength, ECM resilience, articular surface quality, tendon inflammation, bone mineral density, wound healing, microvascular inflammation and muscular strength/size. At present, very little research has been performed to evaluate the practical applications/efficacy of HCOL supplementation in young and/or athletic individuals related to performance and acute recovery from damage.

If the ECM and/or tendon tissue that is intimately linked with skeletal muscle can recover and be returned to optimal strength following damaging exercise, then this could be of practical

benefit to athletes. Furthermore, if HCOL can enhance the effectiveness of ECM during processes involved with the RBE, then MSK tissues may be better protected against subsequent bouts of damaging exercise. Further investigations need to be performed to assess the effectiveness of HCOL supplementation before it can be considered beneficial for healthy or athletic populations. Thus, research investigating the ability of HCOL supplementation to enhance recovery following muscle damage appears to be a logical and important next step.

Chapter 3 - Methodology

The purpose of this study was to assess the effect of HCOL supplementation on acute recovery following a damaging exercise bout in resistance trained males. The hypothesis was that 12-days of HCOL supplementation would be beneficial for resistance trained individuals subjected to an intense bout of eccentric exercise, due to enhanced connective tissue and ECM repair.

Participants

Participants were recruited from the University of Oklahoma and the greater Cleveland County (Oklahoma) area and included males who have been consistently resistance training (≥3 days per week; trained lower body at least once per week) for at least 2 years. Only male participants were selected because it has been shown that females respond less to increases in collagen synthesis after exercise compared to males, as well as possessing lower basal synthesis levels (Miller et al., 2007).

Additionally, participants were between 18-35 years of age, injury free for the previous 12 months, resistance trained and not currently (or within 6 months of the study start date) taking a collagen supplement. If injury/illness occurred during the study period, or the protocol was not adhered to (i.e. not consistently taking the prescribed supplement, or inability to attend testing sessions more than ±1 day from the scheduled date) then the participant was excluded. Vegetarian/vegan individuals were excluded due to the origin of the HCOL supplement.

Due to the similarity in research design, data from Farup et al. (2014b) was used to perform an *a priori* power analysis using G*Power software version 3.1.9.3 (Heinrich Heine, University of Dusseldorf, DE) (Faul et al., 2007). The analysis considered the main performance outcome measure (MVIC) and was based upon a desired statistical power ($1 - \beta$) of 0.80 and a

moderate (Cohen's *d*) effect size of 0.5 (Cohen, 1988). The analysis revealed that 16 subjects (8 per group) were required for this study to be sufficiently powered.

Experimental Design

Fifteen resistance trained males were recruited to participate in a randomized, double blind, placebo controlled trial using a parallel experimental design.

To begin with, participants performed a "Familiarization 1" (F1) session, which involved a full explanation of the protocol and the administration of the Physical Activity Readiness Questionnaire (PARQ), International Physical Activity Questionnaire (IPAQ) short form, health history questionnaire, HIPAA authorization and informed consent documents (requiring signature). Height, weight and body fat percentage using bioelectrical impedance spectroscopy (BIS) (ImpediMed, Queensland, Australia) was recorded. Participants were then familiarized with the VAS scale, the pain pressure threshold (PPT) algometer test, the maximal voluntary isometric contraction (MVIC) test and the countermovement jump (CMJ) test in order to reduce the influence of the learning effect. Following this, participants were familiarized with the muscle damage protocol by performing one practice set of 10 drop jump repetitions. Participants waited 1-2 days before returning for a second familiarization (F2) session, where all the previously mentioned tests were practiced again (with the exception of the muscle damage protocol repetitions). A third and final familiarization session (F3) was performed 1-2 days after F2, where all of the tests were practiced for the final time. During the F3 session, a blood draw was taken to assess pre supplement biomarker measures at the start.

Participants were provided with a randomly assigned supplement to consume daily for the following 12-day period (7-days until muscle damaging protocol + 5 days over the 24h, 48h and 120h post exercise period). They were instructed to consume it at the same time of day

during the 7-day “run in” period, and after completing any testing sessions during the 5-day recovery period. The supplement kit given to participants included 12 individual bags of either 15g (per bag) of Hydrolyzed Collagen peptides (HCOL) (Vital Proteins Collagen Peptides, Chicago, IL, USA) or 15g (per bag) of a cornstarch placebo (PLA) (Argo, ACH Foodservice, IL, USA) as well as a plastic sports bottle to allow the powders to be mixed with 500ml of water as directed. The supplement being provided was blinded to both the researcher administering it, and the participant receiving it. An individual not associated with the current investigation assigned treatments a unique code for future de-identification of its true nature. The coding key related to the treatments, was placed in a sealed envelope until all data was collected and analyzed. All attempts were made to provide highly comparable supplements from a taste and consistency standpoint to reduce the chance of participants being able to “unblind” the supplement. Participants were instructed to consume the supplement once per day, at the same time of day for the 12-day duration of the study. They were instructed to follow their usual dietary and exercise routines, as well as to refrain from taking any new nutritional supplements during this 12-day period. Participants were instructed to perform no lower body exercise in the three days before each testing session and to attend each testing session in an overnight fasted state.

After a 7-day period of supplementation, participants returned to the laboratory for the first testing session (T1). During this session, blood draws, soreness measures, muscular function and jump performance measures were taken before the muscle damaging protocol was performed (PRE) to act as a baseline. The muscle damage protocol was then carried out (Figure 3 below), before all measurements were repeated immediately post (IP), and then 24-hours (24h), 48-hours (48h) and 120-hours (120h) post exercise. The supplement was continually consumed in the post damaging exercise protocol period (i.e. the following 5-days, for 12-days total).

This experimental design allowed us to determine how recovery (muscle and jump performance, soreness and biomarkers) from an acute bout of high-force eccentric exercise would be affected by consuming HCOL. An inert cornstarch placebo was used to account for the placebo effect, as well as to mitigate the impact of variability in free-living habits of participants (i.e. exercise and nutritional habits) during the uncontrolled 7-day supplementation period. To avoid the threat of instrumentation, locations for soreness testing using the algometer were marked, and standardized verbal direction/encouragement was used during the muscle damage protocol and performance measure testing. Furthermore, all efforts were made to test participants before 10:00am, and in most cases at the same time of day each time they returned to the lab. This precaution was taken in order to reduce the influence that circadian rhythm could have on biomarkers and performance measures.

Upon ingestion, plasma levels of Hydroxyproline (a collagen specific amino acid) reach maximum levels after 2 hours, and collagen derivatives have been found in both the free form (amino acid) and peptide form within the blood during a previous investigation by Ohara et al. (2007). Shaw et al. (2017) found an increase in engineered ligament strength after participants consumed gelatin for as little as 3 days. In addition, Clifford et al. (2019) found an improvement in CMJ recovery following ingestion of HCOL whilst using an extremely similar experimental protocol. Thus we feel that the short supplementation period should allow the nutrients sufficient time to interact with intramuscular connective tissues at the least (due to low blood flow and slower metabolic rate of many connective tissues, longer supplementation periods may be required to see changes in these tissues. The inclusion of 24h, 48h and 120h (post baseline) follow up visits allowed us to compare differences in recovery kinetics and indices of physiological damage over the transient acute recovery period.

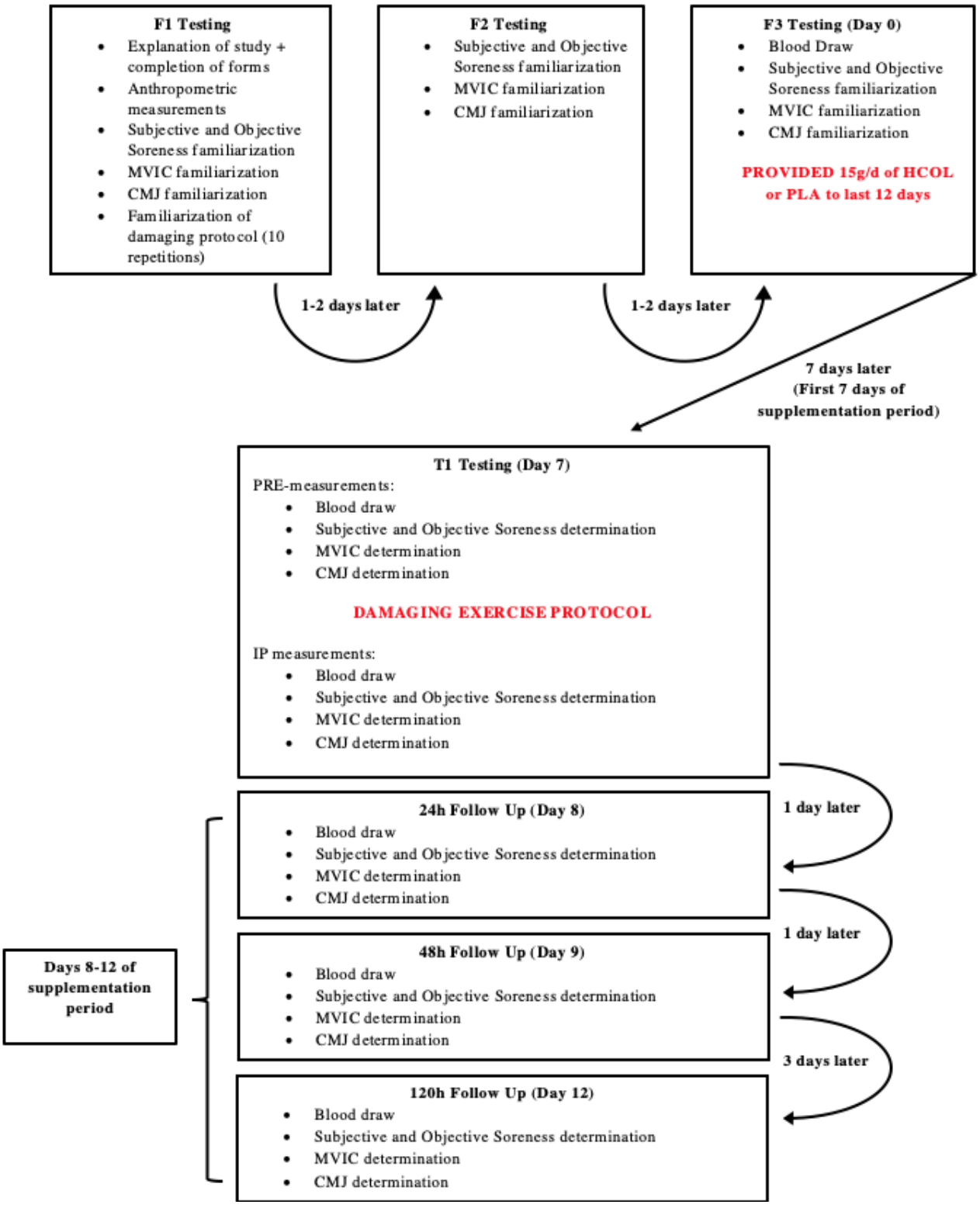


Figure 3. – Visual Representation of Experimental Study Design

Experimental Procedures

Muscle Damage Protocol

Greater levels of muscle damage have been shown to occur using exercise involving an eccentric contraction (Dolezal et al., 2000). In agreement with this, protocols using forced resistive eccentric contractions (Brown et al., 1999), downhill running (Welsh et al., 2014) and plyometric exercise (Tofas et al., 2008) have all been shown to induce acute muscle and connective tissue damage. In order to improve relevance for athletic individuals, a ballistic depth jump protocol utilized during previous investigations was followed (Miyama & Nosaka, 2004; Tofas et al., 2008; Clifford et al., 2019).

An initial warm up was carried out involving light jogging for 5 minutes on a treadmill followed by 20 bodyweight squats. Although a familiarization was performed for the muscle damage protocol during F1, 3 practice repetitions were allowed prior to the performance of the full 100 jump protocol in order to remind participants of correct technique. The complete damaging exercise protocol required participants to perform 5 sets of 20 drop jumps (100 total) with a 2-minute rest in between each set (Miyama & Nosaka, 2004). Participants were required to step from a height of 60cm and land on the floor with both feet whilst keeping their hands on their hips and descending to approximately 90 degrees of knee flexion to emphasize the lower body contribution to landing. They were instructed to counter this landing by performing a maximal vertical jump immediately after reaching the nadir of their initial landing (whilst maintaining hands on hips) in order to better damage tissues involved within the stretch shortening cycle. Participants then used a 30cm step to return to the 60cm drop height ready for the next repetition, where they stepped off with their alternate foot. The participants were instructed to perform this protocol using the same footwear for each measurement session in order to mitigate differences in jumping and landing mechanics/kinetics between sessions.

Recovery from this damage protocol was assessed by comparing a combination of performance measures, pain/soreness measures, and biomarkers.

Recovery – Performance-based Measures

To assess performance, 3 maximal counter movement jumps (CMJ) (Brown et al., 2017) were measured using a calibrated dual force plate (ForceDecks, NMP Technologies, London, UK). Participants were instructed to keep their legs straight, and their hands on their hips whilst in the air (Young et al., 1995). Each maximal CMJ was separated by 30s of rest. Of the 3 CMJs, the jump in which the greatest jump height was achieved was used for data analysis. In addition, quadriceps muscle force was determined using a maximal voluntary isometric contraction (MVIC) for both legs. Whilst seated, participants had inelastic straps wrapped immediately superiorly to their malleoli's and attached securely to a force transducer (model SB-500; Transducer Techniques, Temecula, CA) on a purpose-built chair at the same height. The knee joint angle was fixed at 70°, and checked using a goniometer before each MVIC attempt. Participants received a verbal countdown of 3s before extending their knee "as fast and as hard as possible" (Sahaly et al., 2001), maintaining the contraction for approximately 3s. Verbal encouragement was provided throughout the contraction period. Participants performed 2 isometric MVICs with 120s rest between each effort and the peak force obtained between both trials was used for analysis (Buckley et al., 2010).

Recovery – Pain and Soreness Measures

To evaluate subjective pain/soreness, a 100-mm visual analogue scale (VAS) was used for the total lower body (VAStlbs), as well as specific muscle groups including the quadriceps (VASq), the gluteals (VASg) and the calf (VASc) muscle regions whilst in a resting state. Participants were instructed to draw a line on the VAS scale that described their current level of muscle soreness between the reference points of "worst imaginable pain" and "no pain". To

determine pressure pain threshold (PPT), an analog algometer (Force Dial FDK, Wagner Instruments, Greenwich, Connecticut, USA) was placed at the center of the Vastus Lateralis muscle belly. Participants were placed in a seated position and asked to provide an audible confirmation when the sensation of pressure being applied to the muscle (at an approximate rate of 5 N/s) went from feeling "uncomfortable" to "painful". Measurements were marked with permanent marker to ensure accuracy on consecutive days (Vatine et al., 1993), and two measurements were taken for each leg (30s rest period between consecutive measurements) with the average of the two being used for analysis.

Recovery – Biomarker Measures

Type I Collagen makes up a significant component of the ECM of a number of connective tissues including joints, ligaments and bony structures. To evaluate the response of these tissues to exercise, validated biomarkers have been developed to analyze type I collagen turnover within the body (Kuiper et al., 2005). Procollagen (a precursor molecule to collagen) contains extension peptides at both the amino and carboxy termini, and these Carboxy-terminal Propeptides of Type I Collagen (PICP) are cleaved from the collagen molecule by proteases prior to incorporation into a developing collagen fibril. Increased circulation of PICP represents increased Type 1 collagen synthesis within the body. Degradation of Type I collagen is mediated by two types of proteinases: cysteine proteinases and Matrix Metalloproteinases (MMPs). Carboxy-terminal Telopeptide Region of Type I Collagen (ICTP) is the product of collagen I degradation by MMPs. Although these markers cannot be used to differentiate which tissue the Type I Collagen fragments originated from (i.e. bone/tendon/ECM), these biomarkers have been used previously to assess connective tissue turnover rates in healthy individuals (Kuiper et al., 2005; Paleckis et al., 2015). In addition, measuring both simultaneously and analyzing the ratio

of these biomarkers can provide a more representative estimate of global Type I Collagen metabolism than either in isolation.

Blood samples (7.5mL) were taken from the antecubital vein into evacuated serum separator tubes (SST) for collection. Participants were instructed to fast for 8 hours before the blood draw, and all efforts were made to complete the draws in the morning (07:00-9:30) and at the same time of day for each participant during subsequent testing sessions. Blood was allowed to clot at room temperature for 30 minutes, before being centrifuged (Centra CL3R Refrigerated Centrifuge, Thermo Electron Corporation, Waltham, MA) to separate the serum from the cells. Samples were then aliquoted into 0.5 ml vials and frozen at −80 °C until assays were performed.

On the day of the assay, samples were removed from the freezer one hour prior to the start of the assay to allow for adequate thawing of the serum (all serum analyzed came from samples that had never been thawed before to reduce the chance of protein denaturing from occurring). Commercially available ELISA test kits were used to measure serum concentrations of PICP (CICP/PICP MicroVue, Quidel Corporation, San Diego, CA, USA) and ICTP (ICTP ELISA Kit, Abexxa, Cambridge, UK). All steps detailed in the ELISA kit instruction manuals were followed. For each specified above, two assays were performed (96 wells per kit) and each sample was measured in duplicate. Kit range was determined by calculations defined in the assay instruction manuals because control and ranges were dependent on the type of kit used. In order to test the assay precision, low and high control samples were included in the PICP assay (unfortunately they were not available for the ICTP kit). All control samples were within the reference ranges provided by the manufacturers. For PICP, intra assay %CV's were 3.7-4.3% and the inter assay %CV was 5.1%. For ICTP, intra assay %CV's were 4.1-5.6%, however inter assay was unable to be calculated due to lack of control samples.

Data Analysis

All data are presented as mean ± SD, with an alpha level of $p \leq 0.05$ indicating statistical significance. Normality was determined using a Shapiro-Wilk test. Effect sizes were calculated (Cohen's $d = M_1 - M_2 / \sigma_{pooled}$ where $\sigma_{pooled} = \sqrt{[(\sigma_1^2 + \sigma_2^2) / 2]}$) for any statistically significant result and interpreted according to Cohen (1988), where 0.2 = a small effect, 0.5 = a moderate effect and 0.8 = a large effect. IBM SPSS Statistical Software Version 24 (Armonk, NY, IBM, 2015) was used for all analyses.

An independent samples t-test was performed on all normally distributed baseline measures to ensure homogeneity between groups. A Mann Whitney U test was used for non-normally distributed data (Overall Lower Body Soreness VAS and Left Leg Pain Pressure Threshold at baseline). All biomarkers, VAS soreness, PPT, MVIC and CMJ metrics were analyzed using a mixed model analysis of variance ANOVA with 2 groups (PLA vs COL) and 4 repeated measures time points (PRE, 24h, 48h, 120h). When a significant interaction effect was detected, a post-hoc analysis of the simple effects using a LSD adjustment was used to discover the location of any differences. In the absence of a significant interaction effect, main effects for time and/or group (if found to be statistically significant) were examined using a post-hoc analysis with a LSD adjustment to locate where differences existed. A Levene's test of equal variances was performed to support that the samples for each group were drawn from the same population. A Mauchly's test of Sphericity was used to test that the variances of the differences between all possible pairs of within-subject conditions (i.e. levels of the independent variable) were equal and a Greenhouse-Geisser correction was applied whenever this assumption was found to be violated. A paired sample T-test was used to compare MVIC between the Pre and IP time point in order to determine whether the intensity of the jump protocol was sufficient to

attenuate performance via fatigue. A paired sample T-test was also used to compare PICP and ICTP between the F3 and Pre time points to assess whether changes occurred during the free living supplementation period. Prior to statistical analysis, CMJ metrics and biomarkers with a CV of >15% and outlying data points (>2 SD's outside of the mean) were removed (varied depending on outcome measure, n values for each were reported within figures) .

Chapter 4 – Results & Discussion

Baseline Characteristics

In total, fifteen participants completed the entire study and their physical characteristics at baseline are shown in Table 1. An independent samples t-test determined no significant differences between normally distributed baseline characteristics, however the Mann Whitney U test determined that left leg pain pressure threshold (not normally distributed) was significantly lower in the HCOL group (p=0.029; ES = 0.57).

Table 1. Baseline characteristics of the placebo (PLA; n=8) and collagen (HCOL; n=7) groups reported as mean±SD

		PLA	HCOL
Age (years)		22.3±2.5	23.0±3.4
Weight (kg)		87.1±19.1	93.8±19.4
Height (cm)		175.9±8.3	181.3±7.4
Training Experience (years)		6.0±2.1	6.8±5.0
Body Fat (%)		18.4±7.7	21.2±4.1
Fat Free Mass (kg)		70.1±11.9	73.7±13.2
Quadriceps MVIC (Nm)	**Left**	294±84	313±73
	Right	299±56	310±87
CMJ Height (cm)		38.7±7.8	38.2±9.2
Vastus Lateralis PPT (lbs)	**Left**	20.8±6.3	14.3±1.9*
	Right	20.2±5.4	15.9±3.2
VAS Lower Body Soreness (mm)		11.3±10.1	10.3±10.6
PICP (ng/ml)		199±99	233±140
ICTP (ng/ml)		14.3±4.8	14.5±6.6

* = denotes statistically significant difference between groups (p≤0.05)

Maximal Voluntary Isometric Contraction

Changes in MVIC immediately following the muscle damage protocol were analyzed using a paired samples t-test for each leg and each condition to assess the magnitude of fatigue imposed by the protocol. In the left leg, a significant decline was seen for both the HCOL group (p=0.029; ES=0.32) and PLA group (p=0.013; ES=0.23) (Figure 4). In the right leg, a significant decline was seen for the PLA group (p=0.020; ES=0.22) but not the HCOL group (p=0.091) (Figure 4).

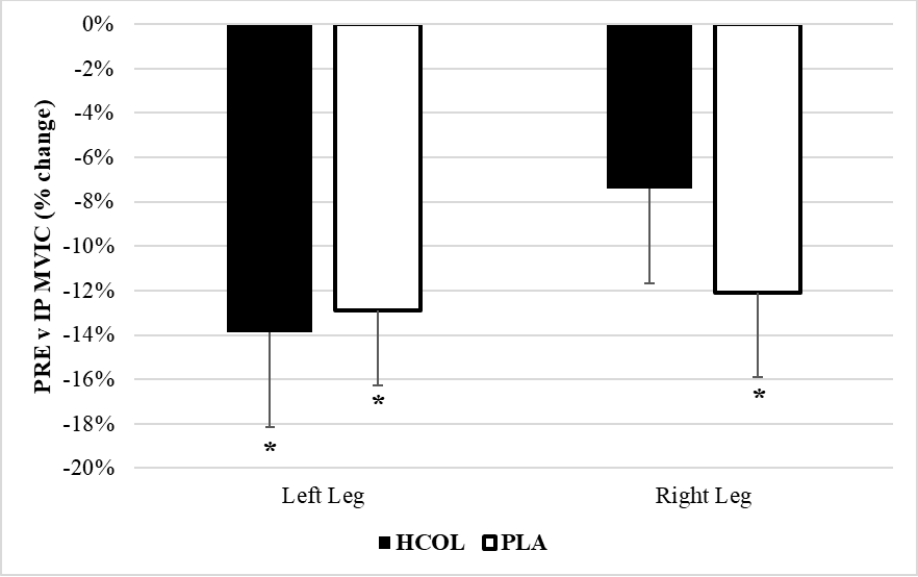


Figure 4. – Percentage differences (mean±SE) in Quadricep MVIC immediately following completion of the muscle damage protocol for the left and right legs in the collagen supplementation group (HCOL; n=7) and the placebo group (PLA; n=8) presente

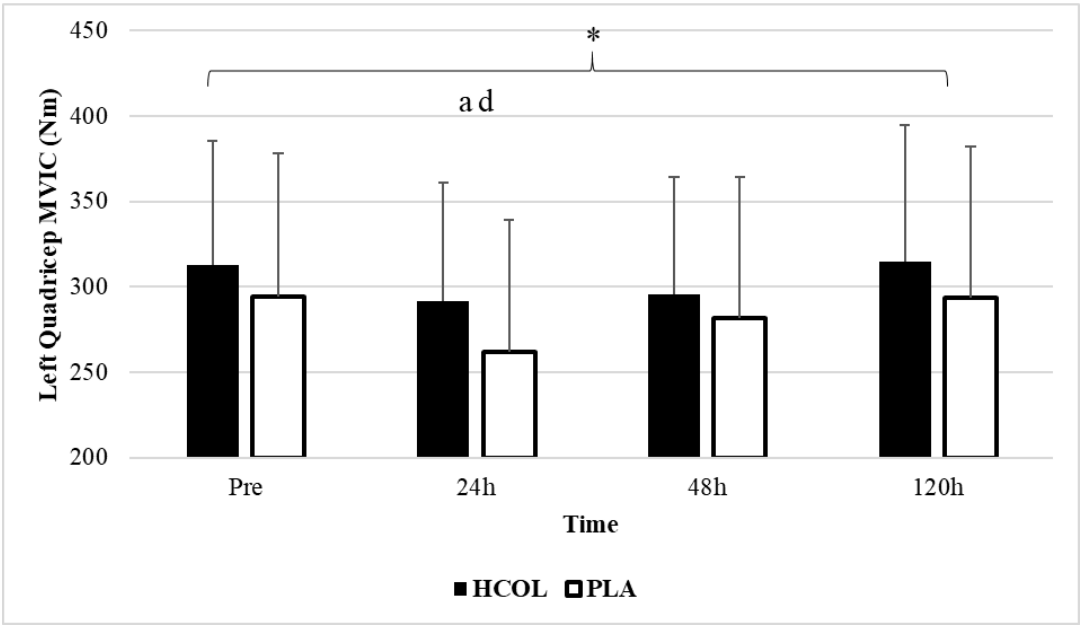
* = denotes statistically significant difference between PRE and IP time points (p≤0.05)

For the raw values, there were no significant group (Left: p=0.614) (Right: p=0.847) or group x time interactions for either leg (Left: p=0.629) (Right: p=0.129), however there was a significant effect for time in both the left (p=0.007) and right (p=0.010) legs over the experimental period. The post-hoc analysis for the left leg MVIC showed that force was significantly lower at the 24h time point (MVIC = 108.7±28.8lbs) compared to the PRE time point (MVIC = 119.4±30.3lbs; p=0.029; ES=0.18) and 120h time point (MVIC = 119.6±32.5lbs;

p=0.029; ES=0.17) (Figure 5i). For the right leg, force was significantly lower at the 24h time point (MVIC = 112.0±26.9lbs) compared to the 120h time point (MVIC = 121.3±29.8; p=0.013; ES=0.16) but not significantly different to PRE (MVIC = 118.9±28.3; p=0.0503 (Figure 6i).

For the left leg percentage change values, post-hoc analysis revealed that the 24h time point (Change v baseline = -8.5±10.5%; p=0.002; ES=0.46) and 48h time point (Change v baseline = -4.5±7.8%; p=0.013; ES=0.34) were significantly different compared to the 120h time point (Change v baseline = 0.1±6.9), as well as a significantly difference between each other (p=0.026; ES=0.21) %) (Figure 5ii). For the right leg percentage change values, the 24h time point (Change v baseline = -4.6±11.7%; p=0.011; ES=0.34) and 48h time point (Change v baseline = -4.1±7.9%; p=0.001; ES=0.40) were also significantly different compared to the 120h time point (Change v baseline = 2.8±8.1%), but not to each other (p=0.932) (Figure 6ii).

i)



ii)

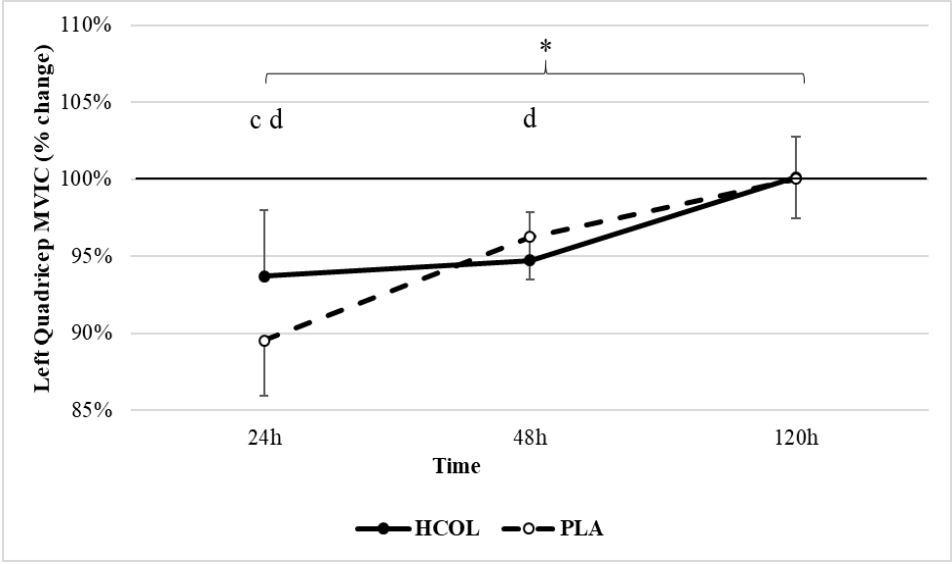


Figure 5. – Differences in Quadricep MVIC for the left leg between the collagen supplementation group (HCOL; n=7) and the placebo group (PLA; n=8) were assessed across four different time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage). i) shows raw MVIC (lbs) scores as mean±SE, ii) shows % change compared to the Pre muscle damage time point (mean±SE)

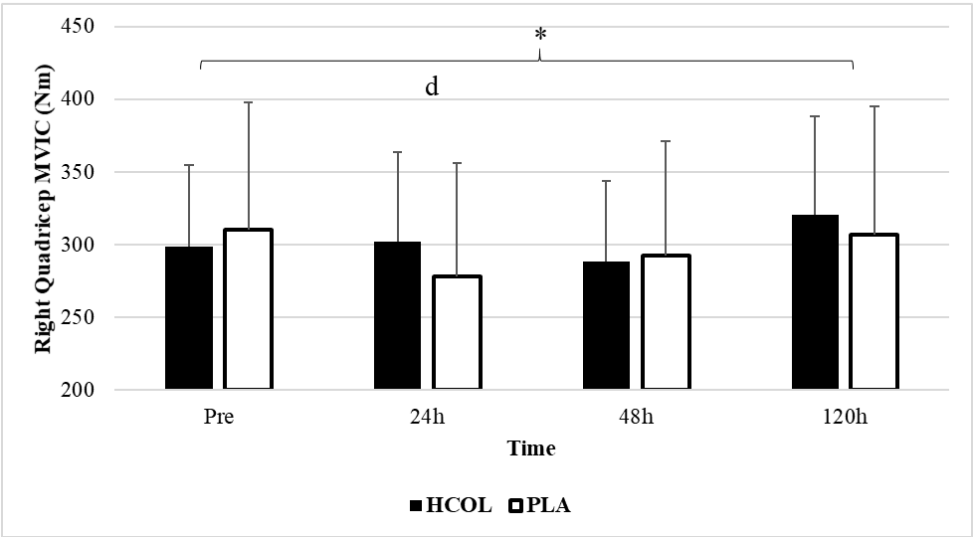
* = denotes significant time effect ($p \leq 0.05$)

a = significantly different from the PRE post muscle damage time point ($p \leq 0.05$).

c = significantly different from the 48h post muscle damage time point ($p \leq 0.05$).

d = significantly different from the 120h post muscle damage time point ($p \leq 0.05$).

i)



ii)

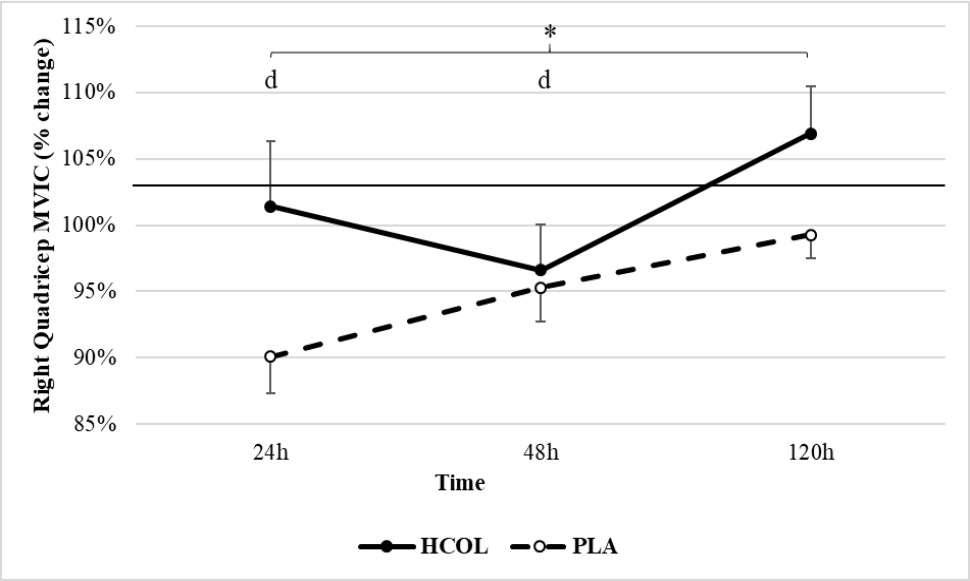


Figure 6. – Differences in Quadricep MVIC for the right leg between the collagen supplementation group (HCOL; n=7) and the placebo group (PLA; n=8) were assessed across four different time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage). i) shows raw MVIC (lbs) scores as mean±SE, ii) shows % change compared to the Pre muscle damage time point (mean±SE)

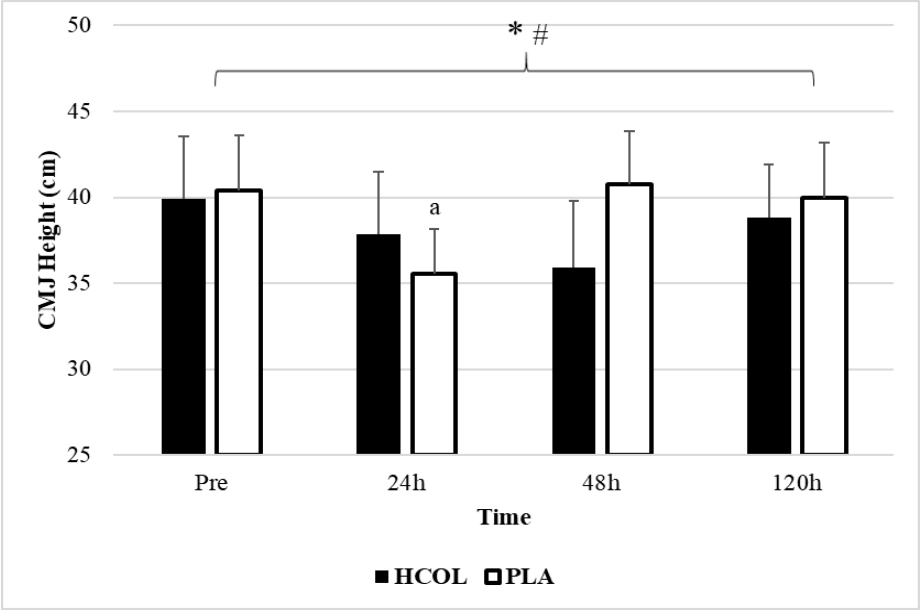
* = denotes significant time effect ($p \leq 0.05$)

d = significantly different from the 120h post muscle damage time point ($p \leq 0.05$).

Countermovement and Drop Jump Performance

For raw CMJ height values, there was a significant interaction effect ($p<0.001$). A post-hoc assessment analyzing the simple time effects revealed that CMJ height was significantly lower than baseline (PRE CMJh = 40.42±7.88cm) for the PLA group at the 24h time point (CMJh = 35.53±6.41cm; $p=0.005$; ES=0.32), but was not significantly different at the 48h (CMJh = 40.77±7.57cm; $p>0.05$) and 120h (CMJh = 39.97±7.84cm; $p>0.05$) time points (Figure 7i). The same post-hoc assessment for the HCOL group found that CMJ height was not significantly different from the baseline score (PRE CMJh = 39.93±8.78cm) at any time point (24h CMJh = 37.85±8.87cm; $p=0.451$) (48h CMJh = 37.10±8.97cm; $p=0.102$) (120h CMJh = 38.85±7.52cm; $p>0.05$) (Figure 7i).

i)



For CMJh percentage change values, the simple effects post-hoc analysis for time revealed that the 24h time point (Change v baseline = -13.8±2.4%; $p<0.001$; ES=0.18) and 48h time point (Change v baseline = -1.0±3.7%; $p=0.001$; ES=0.52) were significantly different compared to the 120h time point (Change v baseline = -2.3±3.5%) in the PLA group (Figure 7ii).

The same test in the HCOL group found that percentage change of CMJ height was only significantly different between the 24h (Change v baseline = -5.4±6.4%; p=0.017; ES=0.16) and 48h time point (Change v baseline = -7.4±6.3%). The simple group effect analysis revealed a significant difference between the HCOL (Change v baseline = -5.4±6.4%) and PLA (Change v baseline = -13.8±2.4%; p=0.023; ES=0.66) at the 24h time point only.

ii)

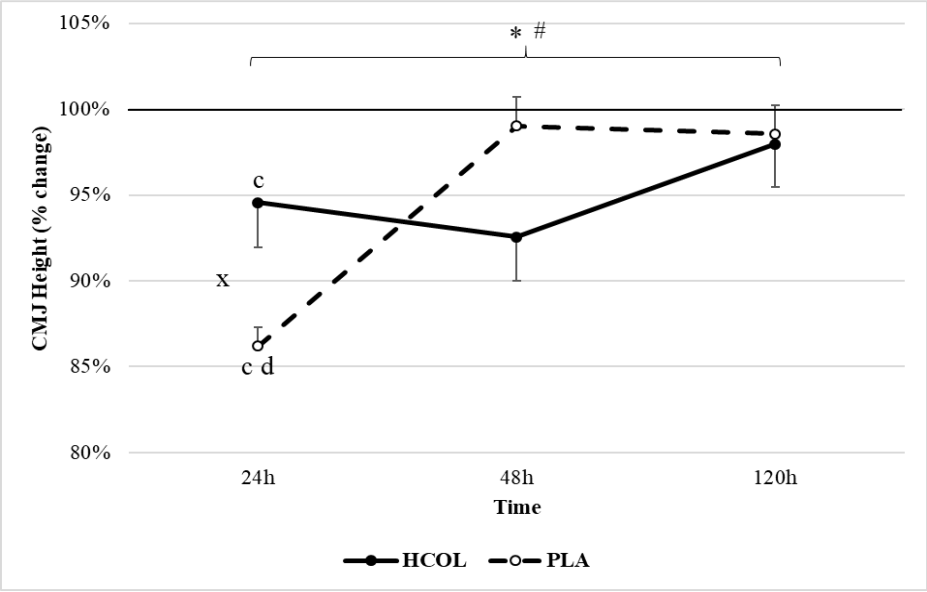


Figure 7. – Differences in counter movement jump (CMJ) height between the collagen supplementation group (HCOL; n=6) and the placebo group (PLA; n=6) were assessed across four different time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage). i) shows raw CMJ height (cm) as mean±SE, ii) shows % change compared to the Pre muscle damage time point (mean±SE)

* = denotes significant time effect (p≤0.05)

= denotes significant interaction effect (p≤0.05)

a = significantly different

from the PRE time point (p≤0.05).

c = significantly different from 48h (p≤0.05)

d = significantly different from 120h (p≤0.05)

x = significantly different from PLA group (p≤0.05)

Individual data points for CMJ performance across the recovery period are presented to demonstrate the variability between responses (Figure 8). Incomplete participant data was removed (i.e. if data was missing for a single time point, the whole line was removed).

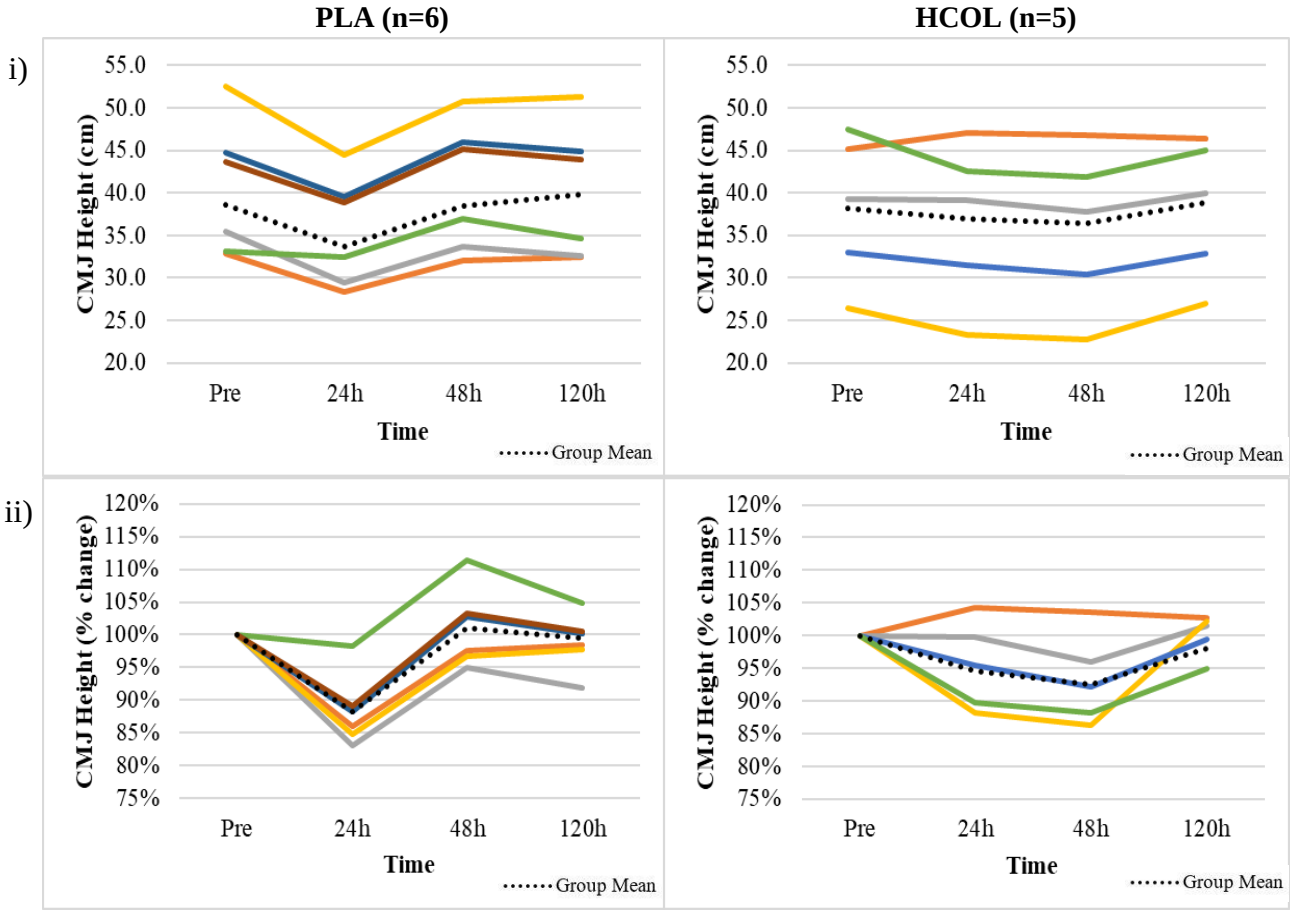


Figure 8. – Individual data points for changes in counter movement jump (CMJ) height across four different time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage). Graphs on the left show data for PLA group and graphs on the right show data for HCOL group. i) shows raw CMJ height (cm) changes, ii) shows % change compared to the Pre muscle damage time point

No significant interactions were found for any of the other CMJ variables analyzed ($p>0.05$), however there was a significant time effect for CMJ RSI-mod ($p=0.019$) and CMJ stiffness ($p=0.050$). A post-hoc analysis determined that at both the 24h ($p=0.047$; ES=0.16) and 48h ($p=0.014$; ES=0.17) time point, scores were significantly lower compared to the 120h time point (Table 2). The post-hoc analysis also revealed that CMJ lower body stiffness was significantly lower at the 24h time point compared to the 120h time point ($p=0.016$; ES=0.22) (Table 2).

Table 2. A number of metrics analyzed during the CMJ trials of the placebo (PLA; n=6) and collagen (HCOL; n=6) groups reported as mean±SD. * = significant time effect ($p\leq0.05$)

		Pre	24h	48h	120h
CMJ Peak Power (W)	PLA	5047±1036	4691±992	5094±1150	4963±1098
	HCOL	4819±881	4736±928	4688±852	4786±782
CMJ Take Off Peak Force (N)	PLA	2057±400	2009±353	2029±385	2023±384
	HCOL	1997±285	2004±329	1976±265	1994±298
CMJ RSI-mod (m/s)	PLA	0.48±0.10	0.40±0.10	0.43±0.09	0.48±0.11
			*		
			d	d	
	HCOL	0.44±0.14	0.44±0.15	0.41±0.14	0.45±0.16
			*		
			d		
CMJ Lower Body Stiffness (N/m)	PLA	5557±3472	4264±1986	5322±2297	5415±2629
	HCOL	4250±1123	3908±1189	3731±1340	4413±1373

d = significantly different from the 120h time point ($p\leq0.05$).

Muscle Soreness and Pain Pressure Threshold

There was no group x time interaction (p=0.525) for overall lower body muscle soreness determined using a 100mm VAS, however a significant time effect was discovered (p<0.001) (Figure 9). The same findings were seen with the VAS scores for individual lower body muscle groups, including the Quadriceps region (Interaction: p=0.575; Time: p=0.006), the Gluteal region (Interaction: p=0.236; Time: p<0.001) and the Calf region (Interaction: p=0.941; Time: p<0.001) (Fig 10). The post-hoc analysis of the time effect revealed that overall lower body muscle soreness was significantly higher than PRE (11 ± 10mm) at both the 24h (43 ± 25mm; p<0.001; ES=0.64) and 48h (32 ± 23mm; p=0.003; ES=0.51) time points, but that it returned to baseline by 120h (11 ± 11mm; p=0.955). A similar pattern was seen in the VAS results for the individual muscle groups, with significant time effects indicated on Figure 10.

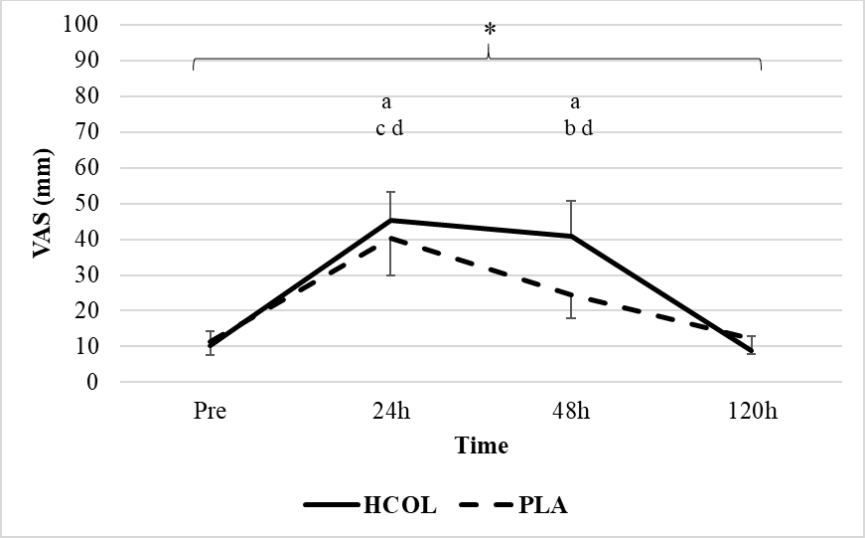


Figure 9. – Differences in subjective muscle soreness for the overall lower body between the collagen supplementation group (HCOL; n=7) and the placebo group (PLA; n=8). Soreness was assessed using a 100mm VAS across four different time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage) and reported as mean±SD.

* = denotes significant time effect (p≤0.05).

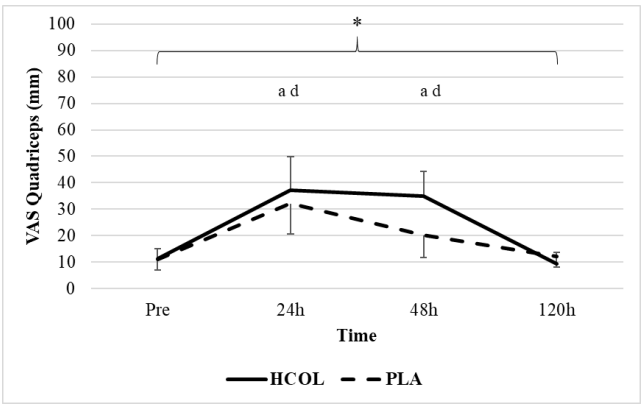
a = significantly different from the PRE muscle damage time point (p≤0.05).

b = significantly different from the 24h post muscle damage time point (p≤0.05).

c = significantly different from the 48h post muscle damage time point (p≤0.05).

d = significantly different from the 120h post muscle damage time point (p≤0.05).

i)



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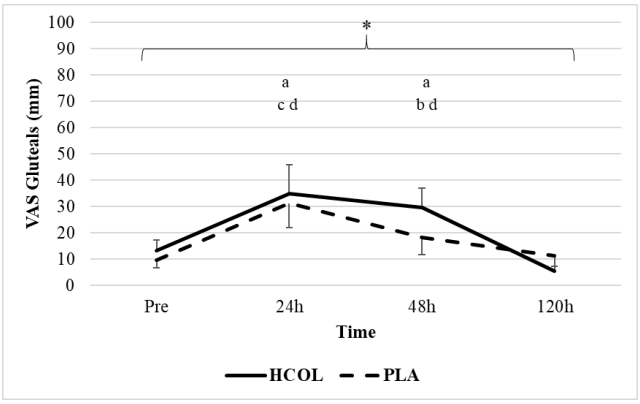


iii)

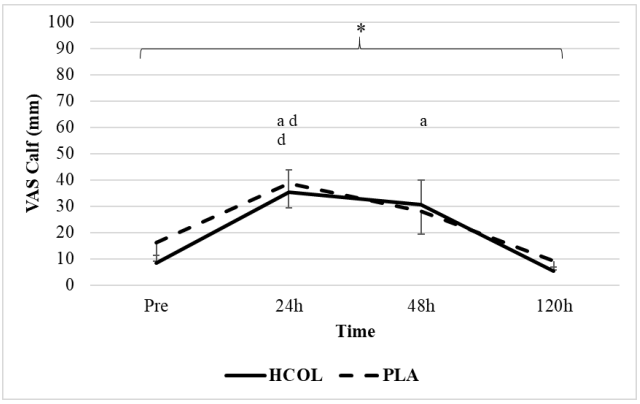


Figure 10. – Differences in subjective muscle soreness for the i) Quadricep region, ii) Gluteal region, and iii) Calf region between the collagen supplementation group (HCOL; n=7) and the placebo group (PLA; n=8). Soreness was assessed using a 100mm VAS across four different time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage) and reported as mean±SE.

* = denotes significant time effect ($p \leq 0.05$).

a = significantly different from the PRE muscle damage time point ($p \leq 0.05$).

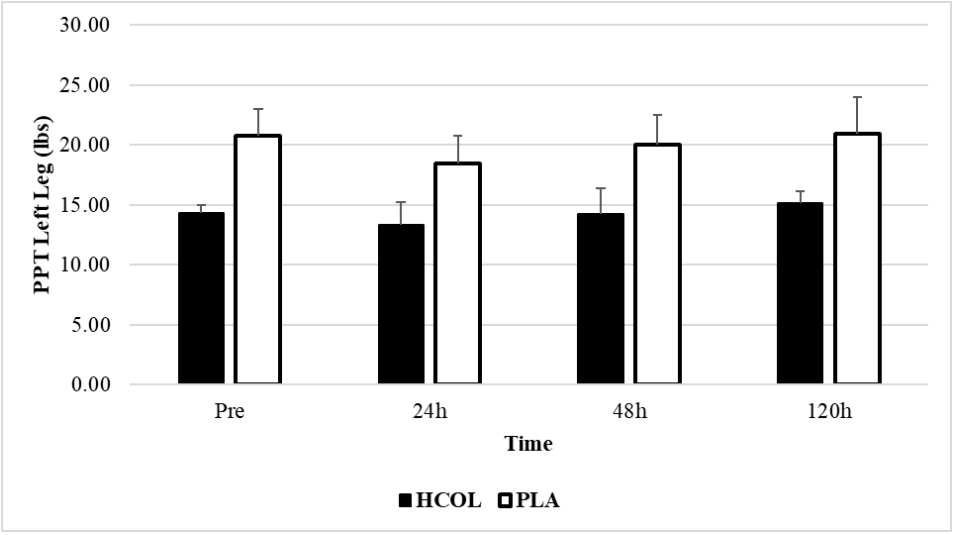
b = significantly different from the 24h post muscle damage time point ($p \leq 0.05$).

c = significantly different from the 48h post muscle damage time point ($p \leq 0.05$).

d = significantly different from the 120h post muscle damage time point ($p \leq 0.05$).

For the pain pressure threshold measurements taken at the Vastus Lateralis, neither a significant time (Left: p=0.280; Right: p=0.321), group (Left: p=0.064; Right: p=0.114) or group x time interaction (Left: p=0.960; Right: p=0.736) was seen (Figures 11 & 12).

i)



ii)

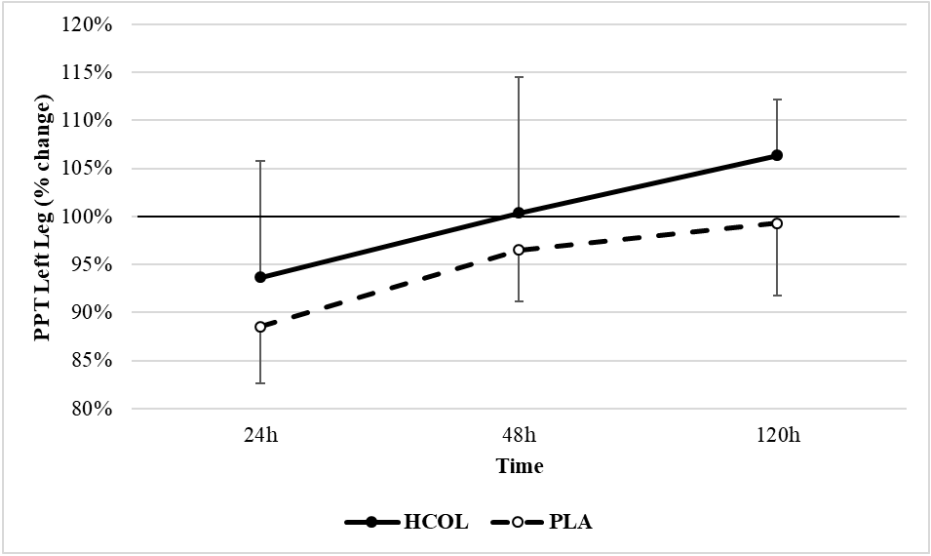
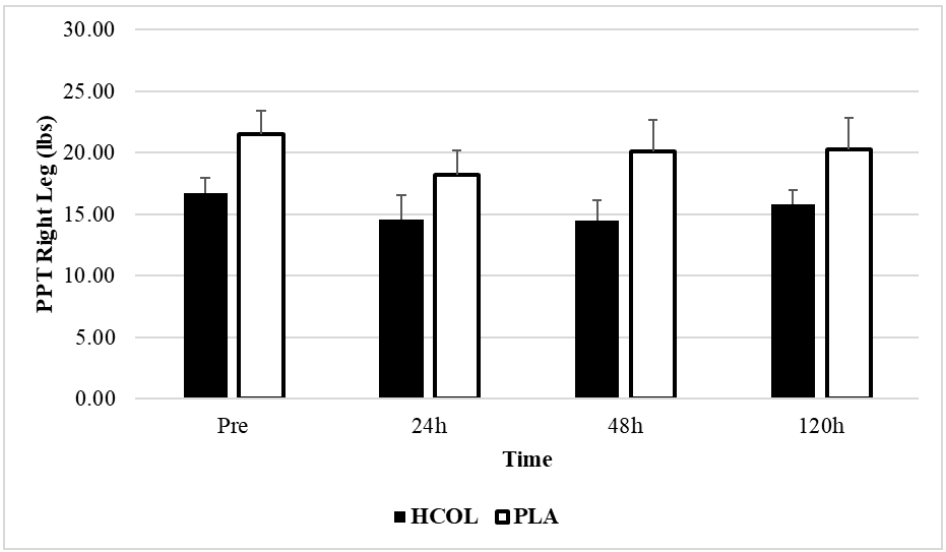


Figure 11. – Differences in left leg pain pressure threshold (PPT) between the collagen supplementation group (HCOL; n=7) and the placebo group (PLA; n=8) were assessed across four different time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage). i) shows raw PPT values (lbs) as mean±SE, ii) shows % change compared to the Pre muscle damage time point (mean±SE)
No significant differences p>0.05

i)



ii)

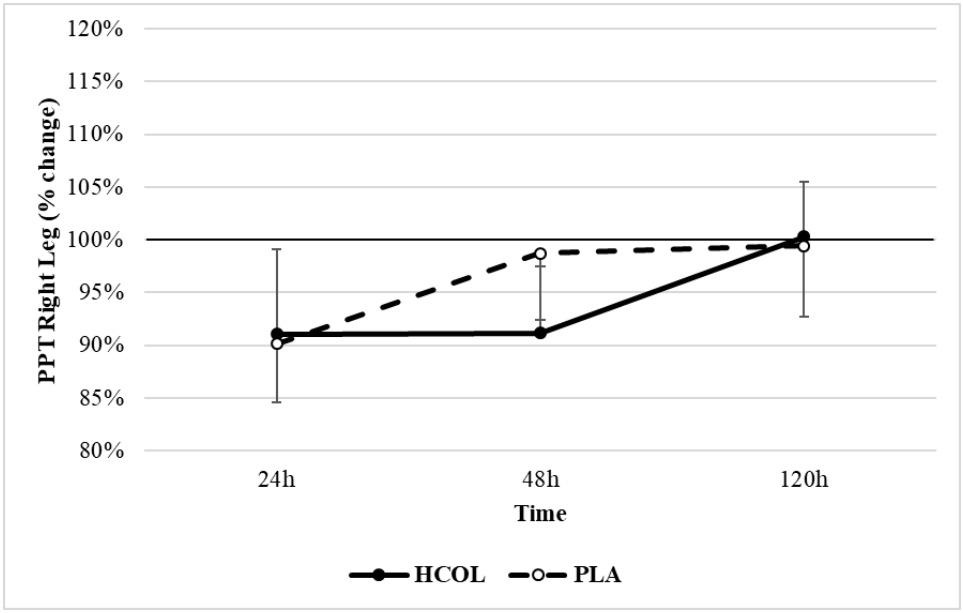


Figure 12. – Differences in right leg pain pressure threshold (PPT) between the collagen supplementation group (HCOL; n=7) and the placebo group (PLA; n=8) were assessed across four different time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage). i) shows raw PPT values (lbs) as mean±SE, ii) shows % change compared to the Pre muscle damage time point (mean±SE)

No significant differences $p>0.05$

Collagen Biomarkers – PICP and ICTP

For PICP, a paired samples t-test found no significant differences in the HCOL (p=0.704) or PLA groups (p=0.138) between the "Familiarization 3" and "Pre" time points (Figure 13). For ICTP, a paired samples t-test also found no significant differences in the HCOL (p=0.224) group between the "Familiarization 3" and "Pre" time points (Figure 14). Due to a lack of assay wells available, PLA concentrations between F3 and Pre were unable to be measured.

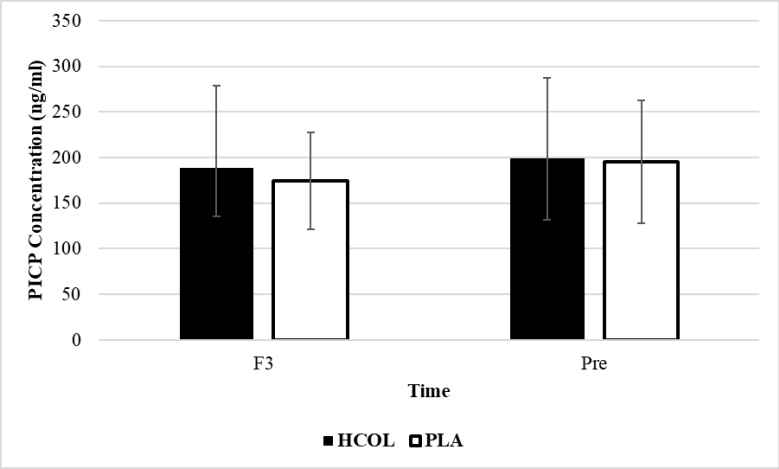


Figure 13. Differences in PICP concentrations (ng/ml) expressed as mean±SD between the Familiarization 3 and Pre time points in the collagen supplementation group (HCOL; n=6) and the placebo group (PLA; n=6).
No significant differences (p>0.05)

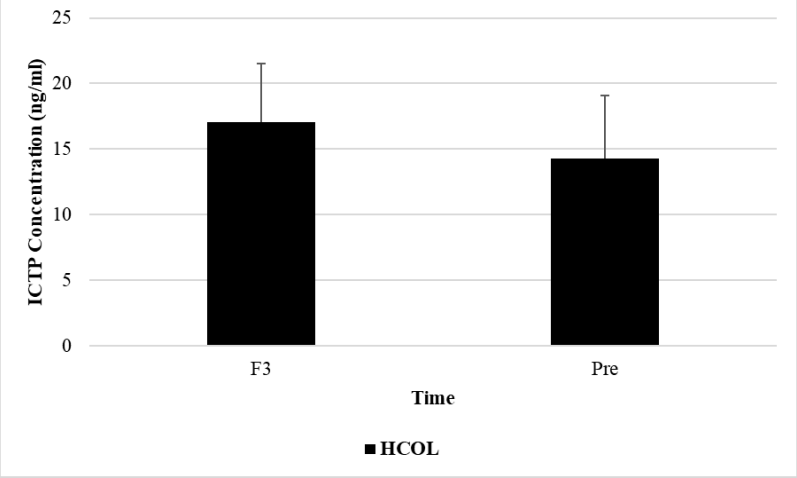


Figure 14. Difference in ICTP concentrations (ng/ml) expressed as mean±SD between the Familiarization 3 and Pre time points in the collagen supplementation group (HCOL; n=6)
(Insufficient assay wells available at Familiarization 3-time point to assess PLA group)
No significant differences (p>0.05)

A mixed model ANOVA comparing raw PICP concentrations between the PLA and HCOL group across the 4 time points did not detect a significant time (p=0.684), group (p=0.813) or interaction effect (p=0.246) (Figure 15i).

In addition, there were no significant time (p=0.215), group (p=0.484), or interaction (p=0.119) effects when PICP was expressed as a percentage change (Figure 15ii).

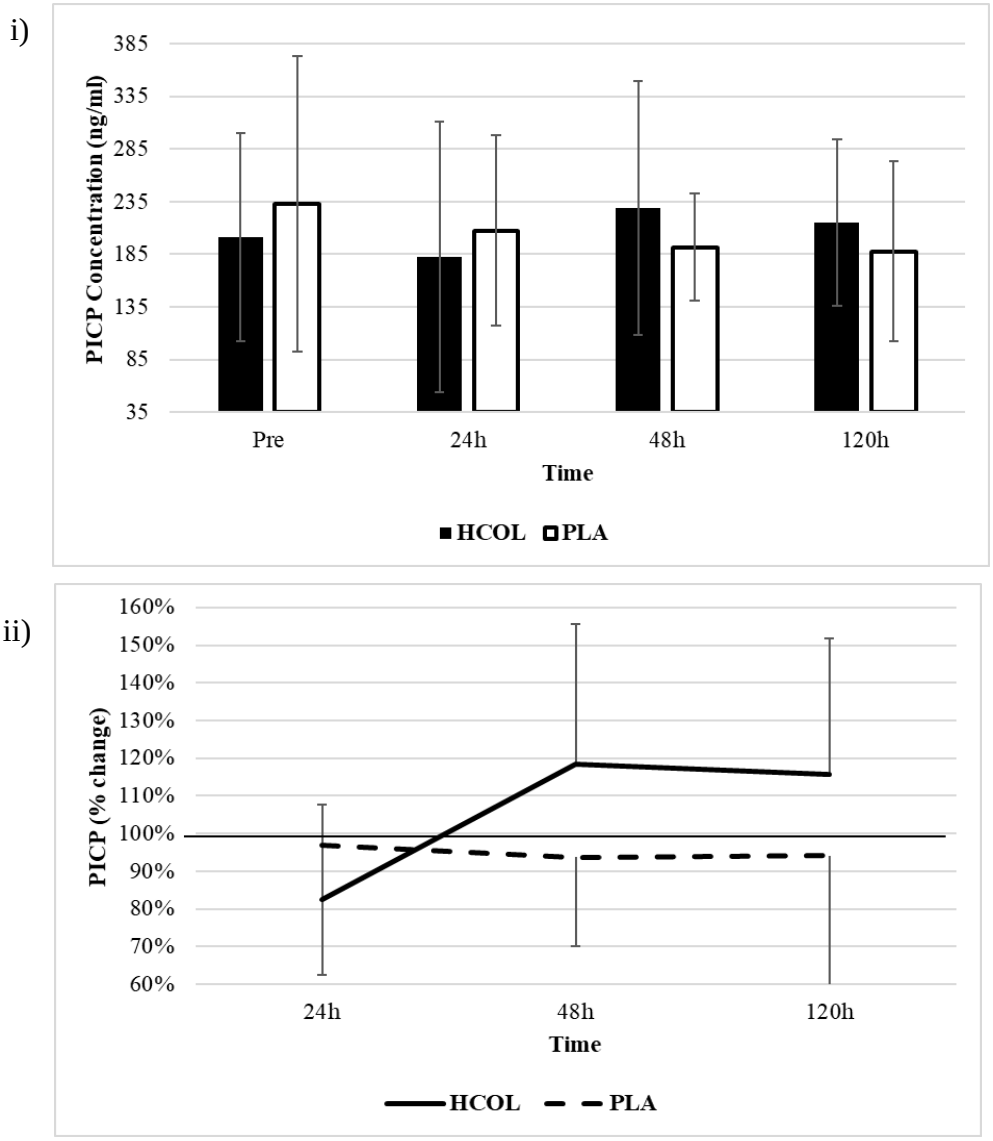


Figure 15. Differences in serum PICP concentrations between the collagen supplementation group (HCOL; n=5) and the placebo group (PLA; n=8) assessed across four time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage). i) shows raw PICP concentrations (ng/ml) as mean±SD, ii) shows % change compared to PRE (mean±SD)
No significant differences detected (p>0.05)

A mixed model ANOVA comparing ICTP concentrations between the PLA and HCOL group across the 4 time points did not detect a significant time (p=0.449), group (p=0.930) or interaction effect (p=0.879) (Figure 16i).

In addition, there were no significant time (p=0.330), group (p=0.797), or interaction (p=0.493) effects when ICTP was expressed as a percentage change (Figure 16ii).

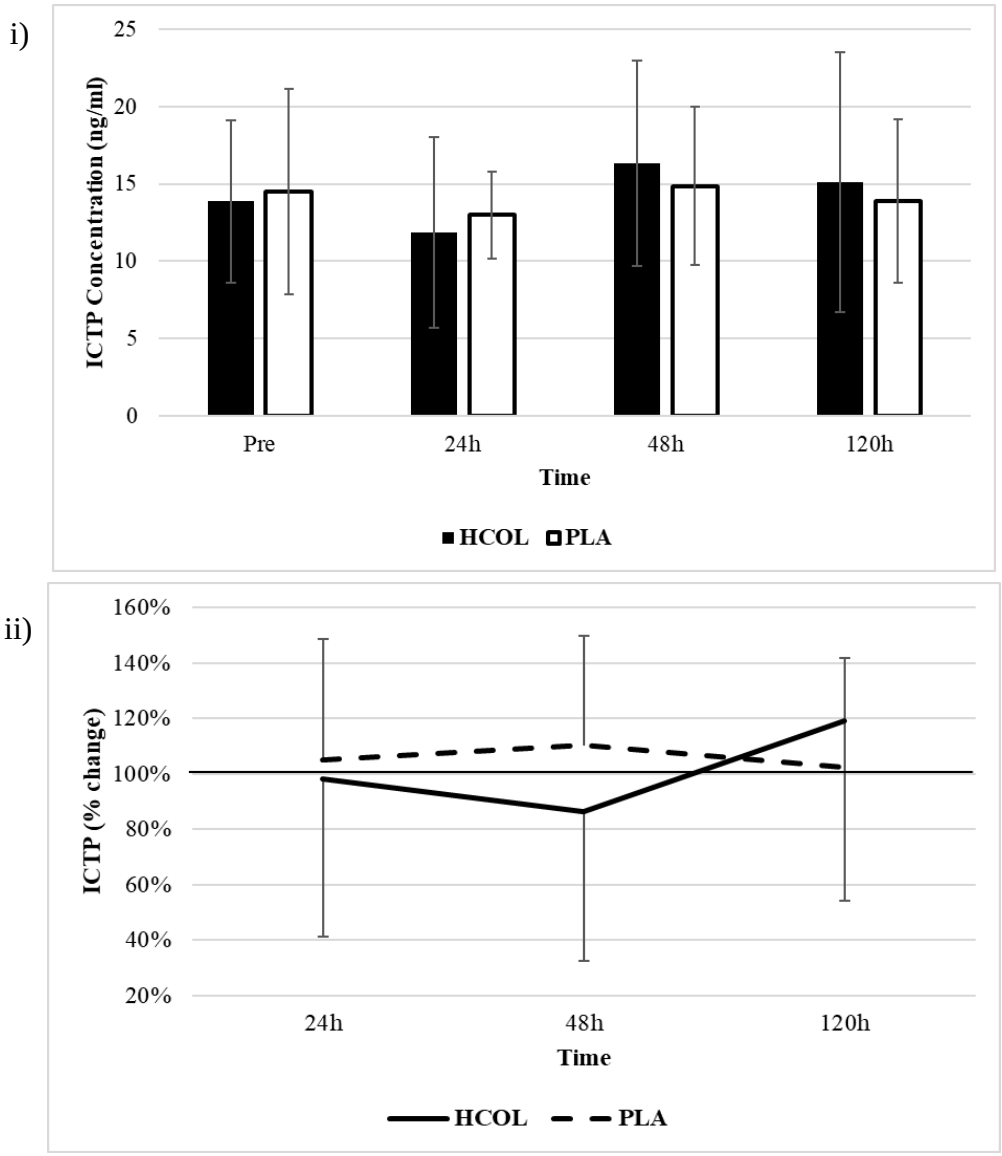


Figure 16. Differences in serum ICTP concentrations between the collagen supplementation group (HCOL; n=6) and the placebo group (PLA; n=5) assessed across four time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage). i) shows raw ICTP concentrations (ng/ml) as mean±SD, ii) shows % change compared to PRE (mean±SD) No significant differences detected (p>0.05)

A mixed model ANOVA comparing serum PICP/ICTP ratio between the PLA and HCOL group across the 4 time points did not detect a significant time (p=0.773), group (p=0.920) or interaction effect (p=0.730) (Figure 17i).

In addition, there were no significant time (p=0.630), group (p=0.274), or interaction (p=0.844) effects when PICP/ICTP was expressed as a percentage change (Figure 17ii).

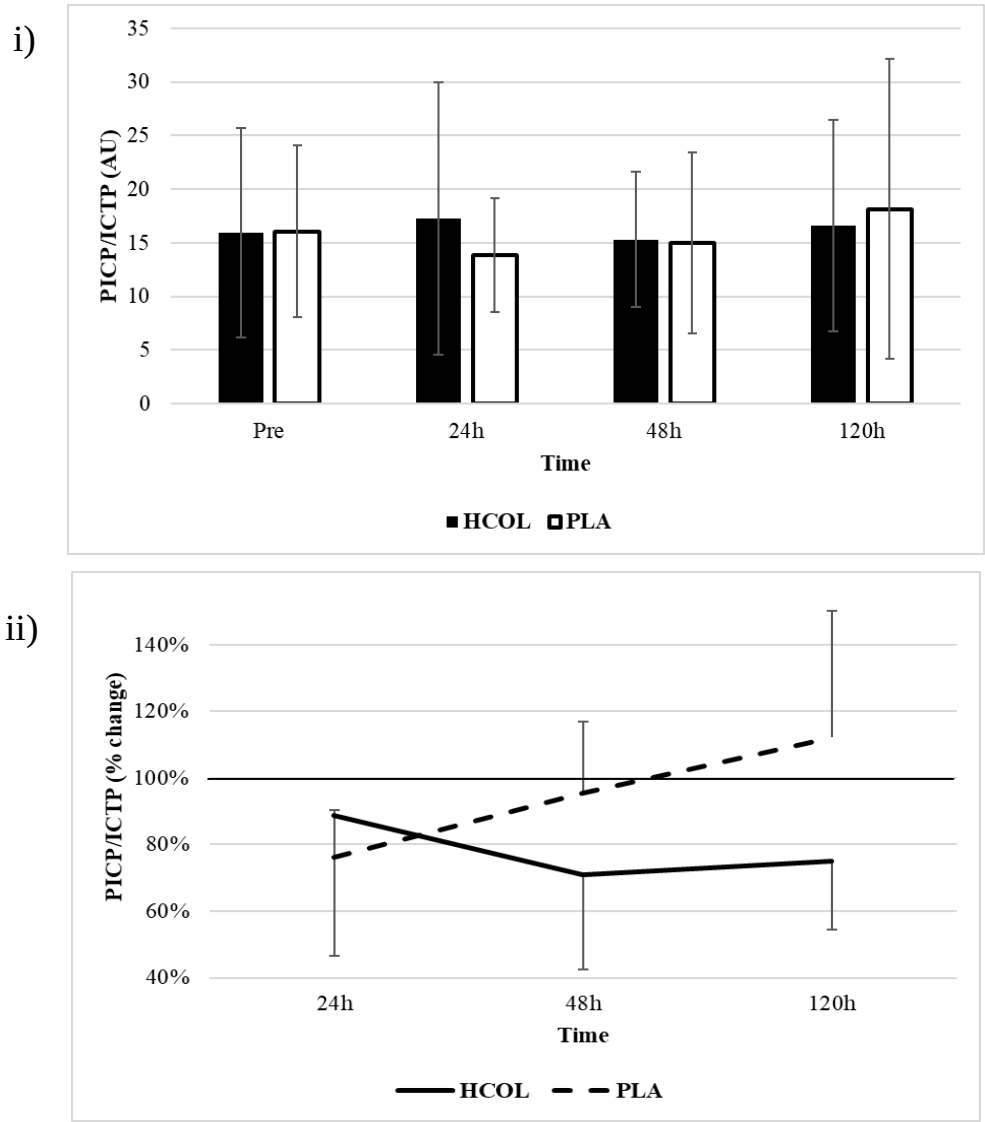


Figure 17. Differences in serum PICP/ICTP ratio between the collagen supplementation group (HCOL; n=4) and the placebo group (PLA; n=6) were assessed across four time points (Pre muscle damage, 24h post muscle damage, 48h post muscle damage and 120h post muscle damage). i) shows serum PICP/ICTP ratio expressed as mean±SD, ii) shows % change compared to the PRE (mean±SD)

Discussion

The purpose of this study was to determine whether consuming a HCOL supplement could provide any meaningful physiological benefits for resistance trained individuals. The experiment was primarily designed to test whether HCOL supplementation could alter the recovery rate of physiological variables (muscular performance, soreness, serum biomarkers) that are typically dysregulated following a bout of damaging exercise. The main findings were: 1) CMJ height performance was significantly decreased at the 24h time point for the PLA group, but not for the HCOL group. 2) The return to baseline (indicating recovery) of every other variable was not significantly different between the HCOL and PLA groups during the 5-day recovery period. 3) Serum PICP and ICTP biomarkers were not significantly altered at any time point in either group.

Muscle Performance (CMJ and MVIC)

The salient finding of this study was that the jumping performance decline that transiently follows a bout of high-force eccentric exercise was attenuated in the HCOL group. At the 24h time point, jump height was significantly decreased in the PLA group but not in the HCOL group. Although some variability is present, Figure 12 seems to show a clear difference in the pattern between individuals in each group in response to muscle damage. The PLA group declined significantly at 24h post but returned to baseline by 48h post and remained there until the 120h time point. In contrast, mean jump height in the HCOL group was not significantly decreased at any time point.

Remodeling and strengthening of the ECM occurs in response to mechanical loading, specifically by increasing tissue collagen content (Mackey et al., 2004) as well as upregulating matricellular de-adhesion proteins and growth factors (Heinemeier et al., 2007; Flück et al., 2008). Furthermore, it has recently been established that the ECM is associated with the

protective adaptations that manifest following a bout of damaging exercise (RBE) (Hyldahl et al., 2015). With novel high-force eccentric contractions, initial muscle fiber damage is followed by a de-adhesive and disassembly response which causes a disorganization of ECM tissue (Mackey et al., 2011). A positive consequence of this initial exposure is a subsequent elevation in ECM anabolic growth factors, collagen synthesis and satellite cell content. Upon re-exposure to the previously novel exercise, a markedly reduced disorganization of the ECM occurs (Mackey et al., 2011).

It seems plausible that consuming HCOL can enhance collagen synthesis within the ECM which may in turn reduce the structural disruption or enhance the effectiveness of the subsequent organizational response. The findings by Lopez et al. (2015) support the hypothesis that HCOL supplementation may confer a protective effect against damage. They provided participants with 3g per day of HCOL or placebo for 6 weeks before having them perform a bench press test (8 sets at 75% of 1RM, 90s rest between sets, repetitions to failure on each set) on day 43, and then again on day 46. Participants in the HCOL group were able to perform more repetitions to failure compared to the PLA group on both day 43 and day 46 (Day 43: HCOL = 57.9% reduction v PLA 72.2% reduction; Day 46: HCOL = 57.8% reduction v PLA = 65.0% reduction). The author's concluded that the HCOL group demonstrated a more robust RBE response and that the supplement may have enhanced the ECM integrity within skeletal muscle. Although the Lopez et al. (2015) investigation employed a longer supplementation period versus the current protocol, the overall quantity of HCOL consumed was similar (125 g total vs. 124 g at 24h). This may point to a total dosage-based relationship versus a duration of supplementation effect on ECM protection.

The difference at the 24h time point could have been the result of HCOL enhancing the repair rate of the intramuscular connective tissues compared to the PLA, or because HCOL attenuated the damage experienced from the eccentric contractions. To test this, a future investigation should perform a follow up testing session at the 8h or 12h post damage period to assess whether jump height performance is attenuated after acute fatigue has dissipated. Regardless of the exact mechanism for this improved performance at 24h, considering the short supplementation period it is most likely that the connective tissues experiencing a positive effect are intramuscular. Connective tissues such as tendons play an important role in transmitting force between the contractile tissue and skeleton, but these tissues receive much less blood flow and have a slower metabolic rate compared to muscle (Baar et al., 2017). Farup et al. (2014b) found that whey protein enhanced patellar tendon thickness when combined with resistance training over a 12-week period. To determine whether HCOL could produce similar effects to whey protein in tendon tissue, using study periods of at least 12-weeks would be prudent.

Clifford et al. (2019) used a similar experimental protocol to us (published concurrently with the manuscript preparation phase of the current study) where they provided 20g per day of HCOL for 9-days and had untrained participants complete 125 drop jumps (6 sets of 25 reps from a 60cm box). They tracked similar outcome measures (VAS soreness, CMJ height, MVIC, biomarkers) 24h and 48h after completion of the muscle damaging protocol but did not observe the same effect that we did at the 24h time point. In our study, participants were resistance trained, so perhaps they were able to absorb or utilize the HCOL more effectively compared with an untrained population. It has long been established that resistance training can increase connective tissue strength (Stone, 1988), so it could also be the case that trained tissues are more readily able to be remodeled/enhanced. Additional research assessing HCOL supplementation

within trained populations is required to confirm this hypothesis. Ideally, a muscle biopsy would be performed to evaluate the local response of different connective tissues, such as intramuscular ECM or tendons.

The pattern of changes in the MVIC results did not seem to match those of the CMJ height results. There was a significant time effect between 24h and 120h but no interaction effect for these measures and Figures 8 and 9 seem to show a different pattern between the groups, mainly at the 24h time point and especially in the right leg. It has been previously suggested that the high individual variability commonly associated with acute recovery studies (Mclester et al., 2003) can be problematic, leading to an inability to detect a significant interaction effect. Another possibility is that due to nature of the exercise, the protective effects of HCOL were not as pronounced. A CMJ involves lengthening (eccentric) and then shortening (concentric) muscle actions, whereas the MVIC involved an isometric contraction, characterized by unchanging muscle length. It has been demonstrated that passive/elastic tissues (e.g. tendons, ECM, titin, actin myosin cross-bridges) within and around the muscle have the potential to store and recover energy, resulting in a profound enhancement of force output (residual force output; RFE) and power production (Roberts, 2016). Acting like a parallel spring, the ECM has been shown to be inherently stiffer than muscle fibers and to substantially contribute towards passive force generation within muscle (Gillies & Lieber, 2011). It is well established that purely isometric contractions generate less force than eccentric contractions, potentially due to reinforcement by titin or other structural proteins within a muscle (Herzog et al., 2016). Therefore, the positive contribution to performance by the series/parallel elastic components may be lower during an MVIC compared to a CMJ. Some data suggests that isometric contractions involve stretching of the associated tendon (Byrne & Eston, 2002), but it is currently unknown how connective tissue

within the muscle itself responds to these contractions. If enhancement of the ECM is the primary mechanism in which HCOL improves performance, then perhaps performance benefits only manifest during movements involving the stretch-shortening cycle to some degree, such as jumping, sprinting or eccentrically biased resistance exercise. It would be interesting to assess whether the protective effect of consuming HCOL manifests itself during different contraction types (isometric v concentric v eccentric) as well as movements with differing ballistic demands (squat jump, sprinting, jumping rope). It is interesting that there were no interaction effects in the other CMJ metrics assessed and it is currently unclear whether other metrics available through the force plate may have showed a better association. Flight time to contraction time has been suggested to better portray recovery/readiness in athletes compared to jump height (Cormack et al., 2008), so analyzing the effect of HCOL on this metric may have value.

We expected to see an improvement in acute recovery of performance measures at 24h or 48h in the HCOL group compared to the PLA group, which was not the case. This was the primary finding in the Clifford et al. (2019) study which utilized a similar design. If consuming HCOL enhance protection and reduced damage incurred by the drop jump protocol, then this may have obscured our ability to detect changes in recovery rate because performance did not decline in the first place. Clinical (Zhang et al., 2008; Kouguchi et al., 2013) and animal models (Vieira et al., 2014; Dar et al., 2017) have shown evidence that consuming HCOL can modulate inflammatory responses. It has been previously suggested that HCOL may enhance acute recovery by altering the inflammatory state within the muscle (Clifford et al., 2019). Other supplements such as tart Montmorency cherry juice seem to enhance recovery from EIMD by interacting with the secondary damage phase (inflammation and reactive oxygen species production) (Owens et al., 2018). We did not evaluate any pro- or anti-inflammatory markers in

this study, thus no conclusions regarding the impact of this mechanism can be confidently made. Further work is required to determine if HCOL can affect any pro- or anti-inflammatory responses that occur in healthy humans following strenuous exercise.

Muscle Soreness

As expected, the drop jump protocol resulted in significant increases in perceived lower body muscle soreness assessed using a VAS scale. Following 100 drop jumps, soreness was elevated at the 24h and 48h time points but had returned to baseline by the 120h time point (termed delayed onset muscle soreness, or DOMS). This seems to be a typical response following high-force eccentric exercise, with DOMS peaking between 1-3 days regardless of the training status of the individuals (Newton et al., 2008; Carvalho et al., 2015). No significant changes occurred over the 5-day time course for soreness assessed using the PPT method. This discrepancy also occurred in the Clifford et al. (2019) experiment and has been previously examined by Lau et al. (2013). They determined that VAS and PPT actually represent different aspects of pain, and that VAS provides a better indication of time course changes for DOMS.

Although the underlying mechanisms of DOMS have yet to be fully elucidated, ultrastructural damage to myofiber filaments and connective tissues, as well as inflammatory processes within the muscle are associated with the phenomenon (Lau et al., 2013). The magnitude of muscle damage that occurs is greater if an exercise bout is eccentric in nature or the individual is unaccustomed to it (Howatson & Van Someren, 2008). We chose to use a drop jump protocol because it achieved both criteria, as well has having greater applicability to real world athletic populations (e.g. soccer, basketball, American football) where landings and/or changes of direction are common.

HCOL supplementation has been shown to reduce joint pain in both OA populations (Kumar et al., 2015), as well as in athletic individuals (Flechsenhar & Alf, 2005; Clark et al.,

2008). It is not yet clear whether it can modulate sensations of pain or soreness within the muscle. Similarly, Clifford et al. (2019) found no significant interaction or group effect in VAS scores in the 24h and 48h following the exercise bout, although their magnitude based inference (MBI) analysis suggested that there may have been a *"possibly beneficial"* and a *"likely beneficial"* effect at 24h and 48h respectively. Lopez et al. (2015) did not report any effect of HCOL on DOMS, although they only provided 3g per day of HCOL to participants and assessed upper body soreness.

Biomarker Response

We did not find a significant group, time or interaction effect for PICP or ICTP in this study. PICP is a protein that is cleaved from a collagen molecule prior to incorporation into a collagen fibril, and is suggested to represent collagen formation within the body. It is similar to PINP (Amino-terminal Propeptides of Type I Collagen), differing only by which end of the Procollagen Type I it is cleaved from (i.e. Carboxyl or Amino terminal ends) (Burtis et al., 2012). ICTP is the product of collagen I degradation by MMPs and represents Type I collagen breakdown throughout the body. These biomarkers have typically been used to assess bone formation/resorption since approximately 90% of the bone matrix is made up of Type I collagen (Burtis et al., 2012). However, serum levels are not sensitive enough to distinguish between the origin of these collagen fragments and have previously been used to evaluate connective tissue status (Kuiper et al., 2005; Langberg et al., 2001). Interestingly, MMPs have been shown to be regulators of the ECM, and to participate in skeletal muscle adaptive modifications caused by exercise (Presti et al., 2017).

Seminal research by Langberg et al. (1999) analyzed PICP and ICTP in both the serum as well as in the peritendinous region of the Achilles tendon (assessed via microdialysis) following completion of a marathon in well trained runners. Compared to resting levels, both serum and

tissue PICP concentrations were significantly decreased during the acute recovery period, but significantly increased by 72h (higher magnitude in tissue; Serum = 24% increase vs. Tissue = 192% increase). They speculated that intensive mechanical loading caused a temporary decrease in collagen synthesis, but that over the following days a positive state of collagen formation was achieved (elevated tissue PICP and unchanged ICTP) (Langberg et al., 1999). Olesen et al. (2007) found a comparable result after participants ran 36km, but cautioned that insertion of the microdialysis probe itself could influence tissue biomarker concentrations. In general, it seems that both acute (Heinemeier et al., 2003; Langberg et al., 2000; Olesen et al., 2007) and chronic (Kuiper et al., 2005) physical activity results in increased PICP concentrations. This biomarker has never been assessed in highly resistance trained populations, so it is unclear how it might respond acutely and chronically to exercise in this population. The mean concentrations we observed were between 189-233ng/ml, which is higher than the reference range concentrations stated in the ELISA instruction manual (69-163ng/ml; based on 279 adults >25 years of age). This suggests that basal collagen synthesis rates are higher in individuals regularly performing resistance training, which may have made it more difficult to detect changes in this group.

Despite being a regularly used biomarker of collagen degradation, the effect of exercise on ICTP is much less consistent. It has been shown to increase (Langberg et al., 2000), decrease (Langberg et al., 1999) and not change (Heinemeier et al., 2003; Langberg et al., 2007; Moerch et al., 2013) in both tissue and serum measurements. Paleckis et al. (2015) had young men perform daily drop jumps (increasing in volume, drop height and squat amplitude every 3 days) but did not see any changes in serum ICTP levels. Thus, ICTP's value as a diagnostic tool for observing changes in collagen metabolism requires further investigation. To our knowledge, this is the first time ICTP has been measured in a population of young, highly resistance trained

males, again making it unclear on how it might respond acutely and chronically to exercise in this population. The mean concentrations we observed were between 10.97-17.04ng/ml, which is higher than mean concentrations typically seen in the literature (usually 2-8ng/ml; no reference range provided in ELISA instruction manual) and suggests that basal collagen degradation rates are also higher in individuals regularly performing resistance training.

We computed the ratio between collagen synthesis/breakdown in order to gain a more complete understanding of collagen metabolism during this experiment. An increased ratio would indicate a potentially beneficial increase in collagen turnover, with implications for connective tissue health and injury prevention/repair. Our data did not show any significant differences despite the pattern seen across time points in Figure 17. It seems that the high inter-individual variability present with both the synthesis (PICP) and degradation (ICTP) biomarkers used to produce the collagen turnover ratio may have obscured the ability to detect any potential changes resulting from the intervention. Kuiper et al. (2005) experienced similar variability during their experiment, so it would be prudent to investigate either methods of reducing this variability, or developing more stable biomarkers for connective tissue assessment.

There is very little data in regard to how HCOL may affect collagen synthesis biomarkers in the body. Shaw et al. (2017) found that providing participants with 15g of gelatin per day for 6 days resulted in a doubling of serum PINP concentrations. They also discovered that engineered ligaments treated with serum from those consuming 15g per day of gelatin had an increased collagen content and tensile strength. In contrast, PINP levels were mostly unaffected by HCOL supplementation in the study by Clifford et al. (2019). The authors suggested that a longer supplementation period may be required for biomarker changes to manifest. This proposal is

further supported by the findings of Konig et al., (2018), where an increase in serum PINP was observed following 12-months of HCOL supplementation in post-menopausal women.

Overall, our data does not support the hypothesis that acute HCOL supplementation influences serum collagen biomarker concentrations. Despite the high-force eccentric contractions, neither collagen synthesis or degradation biomarkers seem to have been altered. It seems likely that the large variabilities we observed were related to sources of biological (circadian, fasting status, prior exercise) or technical (pipetting volume errors, ELISA protocol mistakes, blood sampling procedures) error during serum collection or analysis. Participants were instructed to refrain from eating or exercising before testing sessions, however we cannot be certain that they followed these instructions exactly. PICP concentrations can differ by up to 20% between the peak at 01:30-04:30 and nadir at 11:00-15:00 (Wheater et al., 2013). ICTP has been shown to vary by up to 75% between the peak at 06:00 and nadir at 18:00 (Herrman et al., 2008). Although efforts were made to measure participants between 07:00-09:30, even this 150-minute range could have been affected by circadian rhythm (ICTP in particular). In theory, assessing both collagen synthesis and degradation concentrations would be more useful than either in isolation, thus we feel this approach was warranted. Due to the high variability and loss of power (removal of data points) experienced when analyzing these biomarkers, we were unable to draw conclusions regarding collagen metabolism. Due to technical and financial constraints, it was not possible for us to assess direct markers of collagen synthesis/degradation. Future studies should endeavor to perform peritendinous microdialysis (Miller et al., 2011) or muscle biopsies to assess direct markers of damage and collagen balance within tissues during HCOL supplementation.

Limitations

The primary limitation of this study was that we were unable to assess direct markers of connective tissue synthesis and breakdown. Even if increases in PICP or ICTP had been present, we would have been unable to determine if this was related to the ECM/connective tissue or bone. Due to the occurrence of high inter- and intra-individual variability in biomarker responses (in particular ICTP), some data points from the sample cohort were removed which may have obscured our ability to detect statistically significant differences. To better mitigate this variability, stricter efforts should be made to collect serum at the same time of day for everybody and great sample sizes should be utilized to increase power (especially considering the loss of data that can occur when performing assays).

There is also a possibility that the drop jump protocol did not sufficiently damage the participants. However, considering that we observed a significant reduction in MVIC at both the IP and 24h time points, coupled with a significant increase in muscle soreness in the days following, we believe that sufficient damage was imposed.

Another limitation was that we were unable to control activity or nutrition during the supplementation period. Furthermore, we were unable to get good compliance with food diaries. Despite reminders, the majority of participants never returned their food diaries and the few that were returned were only partially complete. It is possible that diet or protein intake between groups could have influenced the results, although the double-blind randomized nature of the study should have mitigated this effect.

Finally, future studies should attempt to also categorize jumping experience in their participants. Whilst our population contained highly resistance trained individuals, there was some variability in regards to additional exercise bouts that individuals were performing. Although the majority of participants were following a powerlifting style training program, some

were also participating in weekly team sport (i.e. basketball and soccer) or aerobic exercise (i.e. jogging).

Chapter 5 – Conclusion

The purpose of this study was to determine whether consuming a HCOL supplement could be beneficial for well-trained individuals. The three main hypotheses presented were:

1) HCOL supplementation will allow participants to recover from acute bouts of EIMD more rapidly

The return to baseline (indicating recovery) of soreness and performance measures were not significantly different between the HCOL and PLA groups during the 5-day recovery period.

2) 7-days of Hydrolyzed Collagen supplementation will provide a protective effect to the MSK system

CMJ height was significantly decreased at the 24h time point for the PLA group, but not for the HCOL group suggesting that the HCOL may have conferred a protective effect against performance decrements.

3) Serum markers of connective tissue synthesis and breakdown will be significantly and favorably different compared to placebo at either the 24h, 48h, or 120h time period following the muscle damage protocol.

Serum PICP and ICTP biomarkers were not significantly altered at any time point in either group.

Practical Significance and Applications

To our knowledge, this is the first study to suggest that a practical benefit of supplementing with hydrolyzed collagen peptides exists in highly resistance trained individuals. The implications of our findings are that these individuals may be able to reduce the tissue damage and subsequent transient performance decline following a bout of high-force eccentric exercise by employing a preemptive short term period of HCOL supplementation. In athletes,

this may be useful for maintaining the ability to perform during situations in which there is a short time duration between games or during competitions/events spanning several days (i.e. strongman or crossfit competitions, NCAA basketball tournaments). If less damage is performed during the first day of a competition, then physical performance during the following days may be greater. Based on the results of this study, resistance trained individuals who are expecting to experience consecutive days of high-force eccentric exercise may benefit from consuming 15g per day of HCOL in the short term period (7-days) prior to the initial exercise bout.

Future Study Recommendations

Due to the paucity of literature assessing the physiological effects of HCOL supplementation in healthy and/or exercising individuals, future investigations are warranted.

It has been hypothesized that HCOL may interact with connective tissues such as tendons or the ECM. In order to test this hypothesis, future investigations should seek to measure direct markers of tissue damage by utilizing microdialysis or muscle biopsies. The indirect serum markers of connective tissue turnover currently being used during HCOL investigations do not seem to be sensitive or specific enough for researchers to draw valid conclusions regarding the effect that HCOL may have on connective tissues. Observations within animal and clinical populations have suggested that supplemental HCOL may modulate inflammatory pathways. At present, these findings have yet to be observed in healthy/exercising individuals that consume HCOL, however due to the current lack of data, this hypothesis cannot yet be discarded. Thus, future studies should continue to assess changes in pro- and anti-inflammatory biomarkers that may accompany HCOL consumption.

Secondly, to confirm the novel findings of this study, future experiments should expose participants to a secondary bout of damaging exercise in order to elucidate the effect of HCOL

on the RBE. In addition, re-testing jump performance at the 8h or 12h post damage period is recommended to assess whether CMJ height is attenuated after acute fatigue has dissipated in the HCOL group. It may also be of value to assess whether the protective effect of consuming HCOL would still manifest itself during different muscle contraction types as well as exercises involving different ballistic demands.

Thirdly, our findings can only be applied to highly resistance trained males at this time. Future studies should investigate the efficacy of this supplement in alternate populations (females, older individuals, different ethnicities) and ideally in groups with a more homogenous exercise history and/or routine. Specifically, we suggest studying athlete or tactical populations. Not only would we expect these cohorts to exhibit greater homogeneity, but an enhanced protective effect of the MSK system could be of most practical value in such groups.

It has not yet been established how chronic supplementation with HCOL may affect adaptations to training or connective tissue injury prevention/healing rates. Due to the reduced blood flow available to many connective tissues, long term supplementation may be required before benefits can be detected. Investigations of HCOL efficacy within clinical populations have noted benefits with supplementation periods of 12-52 weeks. Future studies should be performed using similar time scales to assess the long term effects of HCOL supplementation on performance/recovery measures.

Finally, our study was developed to test the practical benefits that HCOL could have in exercising individuals. Investigations into the underlying physiological mechanisms that cause these changes are required. Until that information is available, investigators will be forced to speculate on the origin of their findings. A future study employing the measurement techniques

and methodology of Hyldahl et al. (2015), but modified to compare HCOL v PLA conditions would significantly enhance our understanding of how and why HCOL works in humans.

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Appendix 1: IRB Approval Letter

The UNIVERSITY of OKLAHOMA

Institutional Review Board for the Protection of Human Subjects

Initial Submission – Board Approval

Date: October 2, 2018

To: Jason A Campbell, PhD

IRB#: 9698
Meeting Date: 09/10/2018
Approval Date: 10/01/2018
Expiration Date: 08/31/2019

Study Title: Does Hydrolyzed Collagen Supplementation Improve Recovery or Affect Indices of Tissue Damage Following Eccentric Contractions in Resistance Trained Males?

Reference Number: 678669
Study Status: Active - Open

At its regularly scheduled meeting the IRB reviewed the above-referenced research study. Study documents associated with this submission are listed on page 2 of this letter. To review and/or access the submission forms as well as the study documents approved for this submission, open this study from the *My Studies* option, click to open this study, look under Protocol Items to click on the current *Application*, *Informed Consent* and *Other Study Documents*.

If this study required routing through the Office of Research Administration (ORA), you may *not begin your study yet*, as per OUHSC Institutional policy, until the contract through ORA is finalized and signed.

As principal investigator of this research study, it is your responsibility to:

- Conduct the research study in a manner consistent with the requirements of the IRB and federal regulations at 45 CFR 46 and/or 21 CFR 50 and 56.
- Request approval from the IRB prior to implementing any/all modifications.
- Promptly report to the IRB any harm experienced by a participant that is both unanticipated and related per IRB Policy.
- Maintain accurate and complete study records for evaluation by the HRPP quality improvement program and if applicable, inspection by regulatory agencies and/or the study sponsor.
- Promptly submit continuing review documents to the IRB upon notification approximately 60 days prior to the expiration date indicated above.

In addition, it is your responsibility to obtain informed consent and research privacy authorization using the currently approved, stamped forms and retain all original, signed forms, if applicable.

If you have questions about this notification or using iRIS, contact the IRB at 405-271-2045 or irb@ouhsc.edu.

Sincerely,

Karen Beckman, MD, Chair
Institutional Review Board

1105 N. Stonewall Avenue, Oklahoma City, OK 73117 (FWA0007961)

Study documents associated with this submission:

Study Documents			
Title	Version #	Version Date	Outcome
Food Recall Diary	Version 1.0	08/22/2018	Approved
Health History Screen	Version 1.0	08/22/2018	Approved
International Physical Activity Questionnaire	Version 1.0	08/22/2018	Approved
HIPAA Authorization #1	Version 1.0	08/22/2018	Approved
Physical Activity Readiness Questionnaire	Version 1.0	04/19/2018	Approved
Flyer	Version 1.5	08/22/2018	Approved
Email Ad	Version 1.3	08/22/2018	Approved
Protocol	Version 1.5	08/22/2018	Approved

Study Consent Form			
Title	Version #	Version Date	Outcome
Consent Form	Version 1.6	04/19/2018	Approved

Information for Industry Sponsors: the columns titled Version Number and Version Date are specific to the electronic submission system (iRIS) and should not to be confused with information included in the Document and/or Consent title(s).

Appendix 2: Informed Consent Form

Consent Form
University of Oklahoma Health Sciences Center (OUHSC)
University of Oklahoma – Norman Campus
Does Hydrolyzed Collagen Supplementation Improve Recovery or Affect Indices of Tissue Damage Following Eccentric Contractions in Resistance Trained Males?
Principal Investigator: Jay A. Campbell, PhD

This is a research study. Research studies involve only individuals who choose to participate. Please take your time to make your decision. Discuss this with your family and friends.

Why Have I Been Asked To Participate In This Study?
You are being asked to volunteer in a research study being conducted at the University of Oklahoma, within the Health and Exercise Science department. You were selected as a potential participant because you met the criteria of a male who is young, healthy and resistance trained. Please read this form and ask any questions you may have before agreeing to take part in this study.

Why Is This Study Being Done?
The purpose of this study is to see whether a collagen supplement can speed up your recovery rate after an intense exercise session. In additions, we will be looking to see whether this supplement causes any change in muscle or connective tissue damage after this exercise.

What is the Status of the Drugs (Devices or Procedures) Involved in this Study?
The supplement is hydrolyzed collagen (aka collagen peptides). Initially, collagen is extracted from the connective tissue of animals before being subjected to a number of manufacturing processes, such as enzymatic hydrolysis, sterilization and drying. Collagen peptides are the active ingredient found in gelatin (jello). Clinical trials have confirmed that collagen is a safe and well tolerated therapy and the Food and Drug Administration (FDA) center for food safety and nutrition recognize collagen as a product safe for human consumption.

How Many People Will Take Part In The Study?
Up to 26 men may take part in this study; all will participate at this location.

What Is Involved In The Study?
Visit 1 (Familiarization 1) ~ 60 minutes: this will occur at the Department of Health and Exercise Science (face to face meeting) and begin with you filling out a number of forms as well as practicing some of the tests that will be used (this is known as familiarization). The specific tasks to be completed are as follows:

A. Informed consent – you will have the study purpose, study protocol, risks, benefits, protections for privacy and confidentiality, and explanation of the volunteer nature of participation explained to you, along with any questions you may have regarding the consent form. Once you are fully clear about your desire to participate, you will be asked to sign the informed consent form prior to any collection of data.

B. Risk and physical activity assessments – you will be asked to complete the Physical Activity Readiness Questionnaire (PAR-Q), the International Physical Activity Questionnaire (IPAQ), and the AHA/AMA Medical History Questionnaire in order for us to determine your activity levels and appropriateness to participate in the study.

C. Height, weight, age, body fat percentage and occupational status will be obtained/measured.

D. Jump test familiarization - you will practice the test protocol for the countermovement jump and reactive strength index in order to ensure correct technique and reduce the chance of improvements happening just due to more practice (i.e. learning effect).

F. Isometric strength familiarization – you will practice performing the maximal voluntary isometric contraction test in order to ensure correct technique and reduce the impact of the learning effect.

E. Soreness measurement familiarization - you will practice having your soreness measured by using a subjective visual analog scale and also using a pain pressure threshold to ensure you have a clear understanding of what is being asked and to reduce the impact of the learning effect.

H. Eccentric exercise protocol familiarization - you will practice the eccentric exercise protocol being used to incur temporary muscle damage. This will consist of 20 depth jump repetitions from a 60cm box (in the real protocol, you will perform 5 sets of these 20 depth jumps). This will allow us to assess your technique during jumping and landing, and to also allow you to get an idea of what the full protocol will feel like.

Visit 2 = Familiarization 2 – (30 minutes, 1-2 days later) – you will again practice the jump tests, maximal isometric contraction test and soreness tests.

Visit 3 = Familiarization 3 – (30 minutes, 1-2 days later) - you will again practice the jump tests, maximal isometric contraction test and soreness tests. In addition, we will take a blood sample from your arm and a finger prick sample from your finger to provide us with a baseline (pre-supplement) measurement of your blood markers. A trained phlebotomist will be taking all samples to ensure this process is as fast and painless as possible.

You will be randomized to receive either hydrolyzed collagen or placebo (inactive substance, which will look like the study supplement). Randomization means that you have a 50/50 chance of being put into either group. A computer program simulating a flip of a coin will make this random assignment. Neither you nor the researcher will choose which group you will be in, nor will they know which group you have been assigned to.

Before leaving on visit 3, you will be provided with a 12-day supply of either the supplement or placebo powder (12x15g daily servings). You will also be instructed in how to complete a 4-day food diary beginning the day prior to your main testing day.

Visit 4 = Testing Day 1 – (90 minutes, 7 days later) – after taking the supplement that we provided for 7-days, you will return to the lab.

Firstly, you will have another sample of blood taken from your arm and finger. You will then you're your soreness measured, followed by the jump and isometric strength tests that were practiced during the familiarization sessions.

Following this, you will perform the full exercise damage protocol (5 sets of 20 depth jumps from 60cm box, 2-minute rest between sets).

Immediately following the completion of this protocol, all measurements will be re-tested (blood sample, jump measurements, soreness measurements, isometric strength measurement).

You will be instructed to continue taking the supplement and recording food intake during the 48h follow up period.

Visit 5 = 24h follow up – (30 minutes. 1 day later) - you will return to the lab the next day at around the same time of day to have all measurements taken again (blood draw, jump measurements, soreness measurements, isometric strength measurement)

Visit 6 = 48h follow up – (30 minutes. 1 day later) - you will again return to the lab the next day at around the same time of day to have all measurements taken (blood draw, jump measurements, soreness measurements, isometric strength measurement)

Visit 7 = 120h follow up – (30 minutes. 3 days later) - you will return to the lab at around the same time of day to have all measurements taken for the final time (blood draw, jump measurements, soreness measurements, isometric strength measurement)

You will be asked to return the completed food diary on this visit.

How Long Will I Be In The Study?

We think that you will be in the study for between 15-17 days (depending on how many days are taken between familiarization sessions) for a total of 7 visits and approximately 5 hours total. Your participation in the study will be divided into two components:

1) The 3 visits used for familiarization and fitness testing:

Visit 1 (~ 60 minutes)
Visit 2 (~ 30 minutes)
Visit 3 (~ 30 minutes

2) The 4 visits to assess acute recovery:

Visit 4 (~ 90 minutes)
Visit 5 (~ 30minutes)
Visit 6 (~ 30 minutes)
Visit 7 (~ 30 minutes)

There may be anticipated circumstances under which your participation may be terminated by the investigator without regard to your consent.

- He/She feels that it is in your medical best interest.
- You fail to follow study requirements.

You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first.

What Are The Risks of The Study?
Risks and side effects related to the supplement we are studying and protocols we are using are minimal but may include:

- You might experience moderate to severe soreness that is typical of eccentric muscle contractions of an unfamiliar nature, but most of this soreness would be localized to the lower body area.
- You might suffer a lower body injury during the jumping tasks or exercise protocol. All efforts will be made to teach and enforce correct technique to avoid this outcome. If you have suffered any previous lower body injuries that may be aggravated by a landing task, please notify the researcher immediately.
- You experience a reaction to the supplement, however the chances of this occurring are minimal. If you have ever had any adverse reactions to jello, please notify the researched immediately. If you notice any strange effects after consuming the supplement provided, please stop taking it immediately and consider seeing your doctor if symptoms persist.

Are There Benefits to Taking Part in The Study?
The benefits of this study are that you will get your body fat percentage, leg strength and leg power measured accurately. There are no medical benefits for participation.

We hope that the information learned from this study will benefit other individuals in your demographic in the future.

What about Confidentiality?
Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations outside the OUHSC that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the US Food & Drug Administration and other regulatory agencies. The OUHSC Human Research Participant Program office, the OUHSC Institutional Review Board, and the OUHSC Office of Compliance may also inspect and/or copy your research records for these purposes.

What Are the Costs?
There is no cost to you if you participate in this study.

Will I Be Paid For Participating in This Study?
You will not receive monetary compensation for completing this study.

What if I am Injured or Become Ill while Participating in this Study?
In the case of injury or illness results from this study, emergency medical treatment is available.

You or your insurance may be charged for this treatment.

Complications arising as a result of the natural progression of an underlying or pre-existing condition will be billed to you or your insurance. Please check with the investigator or with your insurance company if you have questions.

No other funds have been set aside by the University of Oklahoma or the University of Oklahoma Health Sciences Center to compensate you in the event of injury, illness, or for other damages related to your event of injury or illness.

What Are My Rights As a Participant?
Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled.

If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. You may discontinue your participation at any time without penalty or loss of benefits to which you are otherwise entitled.

We will provide you with any significant new findings developed during the course of the research that may affect your health, welfare, or willingness to continue your participation in this study. You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished. You consent to this temporary restriction.

Whom Do I Call If I have Questions or Problems?
If you have questions, concerns or complaints about the research or have experienced a research-related injury, contact Joel Prowting at joel.prowting@ou.edu or 702-882-9152 OR Dr. Jay A. Campbell at jcampbell21@ou.edu or at 205-435-1935, 24 hours per day, 7 days per week.

If you cannot reach the Investigator or wish to speak to someone other than the investigator, contact the OUHSC Director, Office of Human Research Participant Protection, at 405-271-2045.

For questions about your rights as a research participant, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

Signature:
By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

PARTICIPANT SIGNATURE (age ≥18)	Printed Name	Date
SIGNATURE OF PERSON OBTAINING CONSENT	Printed Name	Date

Appendix 3: HIPAA

University of Oklahoma Health Sciences CenterResearch Privacy Form 1
PHI Research Authorization

AUTHORIZATION TO USE or SHARE
HEALTH INFORMATION[1] THAT IDENTIFIES YOU FOR RESEARCH
An Informed Consent Document for Research Participation may also be required.
Form 2 must be used for research involving psychotherapy notes.

Title of Research Project: **DOES HYDROLYZED COLLAGEN SUPPLEMENTATION IMPROVE RECOVERY OR ALTER INDICES OF TISSUE DAMAGE FOLLOWING ECCENTRIC EXERCISE IN RESISTANCE TRAINED MALES?**

Leader of Research Team: **Dr Jason Allen Campbell**

Address: **1401 Asp Avenue, Room 109, Norman, OK 73071**

Phone Number**: 205-435-1935**

If you decide to sign this document, University of Oklahoma Health Sciences Center (OUHSC) researchers may use or share information that identifies you (protected health information) for their research. Protected health information will be called PHI in this document.

PHI To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your PHI. If you give permission, the researchers may use or share with the people identified in this Authorization any PHI related to this research from your medical records and from any test results. Information used or shared may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form; medical records and charts; name, address, telephone number, date of birth, race, government-issued identification numbers, and fitness testing information such as muscle strength/power testing, body composition testing, and biomarker levels.

Purposes for Using or Sharing PHI. If you give permission, the researchers may use your PHI to evaluate risk of adverse effects occurring during testing and to assess the effect of hydrolyzed collagen supplementation on acute recovery and indices of muscle/connective tissue damage following a bout of intense eccentric exercise in resistance trained males.

Other Use and Sharing of PHI. If you give permission, the researchers may also use your PHI to develop new procedures or commercial products. They may share your PHI with other researchers, the research sponsor and its agents, the OUHSC Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS), and when required by law. The researchers may also share your PHI with collaborating researchers or teams at other institutions.

[1] **Protected Health Information includes all identifiable information relating to any aspect of an individual's health whether past, present or future, created or maintained by a Covered Entity.**

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. The law does not require everyone receiving the information covered by this document to keep it confidential, so they could release it to others, and federal law may no longer protect it.

YOU UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING A COMMUNICABLE OR NONCOMMUNICABLE DISEASE.

Voluntary Choice. The choice to give OUHSC researchers permission to use or share your PHI for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OUHSC researchers to use or share your PHI if you want to participate in the research and, if you cancel your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care unrelated to this study from OUHSC.

Canceling Permission. If you give the OUHSC researchers permission to use or share your PHI, you have a right to cancel your permission whenever you want. However, canceling your permission will not apply to information that the researchers have already used, relied on, or shared or to information necessary to maintain the reliability or integrity of this research.

End of Permission. Unless you cancel it, permission for OUHSC researchers to use or share your PHI for their research will end on February 28, 2021.

Contacting OUHSC: You may find out if your PHI has been shared, get a copy of your PHI, or cancel your permission at any time by writing to:

Privacy Official University of Oklahoma Health Sciences Center PO Box 26901 Oklahoma City, OK 73190	or	Privacy Board University of Oklahoma Health Sciences Center PO Box 26901 Oklahoma City, OK 73190

If you have questions, call: (405) 271-2511 or (405) 271-2045.

Access to Information. You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study is completely finished. You consent to this temporary restriction.

Giving Permission. By signing this form, you give OUHSC and OUHSC's researchers led by the Research Team Leader permission to share your PHI for the research project listed at the top of this form.

University of Oklahoma Health Sciences CenterResearch Privacy Form 1
PHI Research Authorization

Patient/Participant Name (Print): _____

_____ _____
Signature of Patient-Participant Date
or Parent if Participant is a minor

Or

_____ _____
Signature of Legal Representative** Date

**If signed by a Legal Representative of the Patient-Participant, provide a description of the relationship to the Patient-Participant and the authority to act as Legal Representative:

_____
OUHSC may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Patient-Participant or the Legal Representative at the time this signed form is provided to the researcher or his representative.

Appendix 4: Health History Screen

OU Department of Health and Exercise Science

Health Status Questionnaire

Instructions Complete each question accurately. All information provided is confidential.
(NOTE: The following codes are for office use only: RF; MC; SLA; SEP)

Part 1. Information about the individual

1._____
Date

2. _____
Legal name Nickname

3._____
Mailing address

_____
Home phone Business phone

4.Gender (circle one): Female Male (RF)

5. Year of birth: _____ Age _____

6. Number of hours worked per week: Less than 20 20-40 41-60 Over 60

(SLA) More than 25% of time spent on job (circle all that apply)

Sitting at desk Lifting or carrying loads Standing Walking Driving

Part 2. Medical history

7. (RF) Circle any who died of heart attack before age 50:

Father Mother Brother Sister Grandparent

8.Date of: Last medical physical exam: _____Last physical fitness test: _____
Year Year

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9. Circle operations you have had:

Back (SLA)	Heart (MC)	Kidney (SLA)	Eyes (SLA)	Joint (SLA)	Neck (SLA)
Ears (SLA)	Hernia (SLA)	Lung (SLA)	Other _____		

10. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

Alcoholism (SEP)	Diabetes (SEP)	Kidney problem (MC)
Anemia, sickle cell (SEP)	Emphysema (SEP)	Mental illness (SEP)
Anemia, other (SEP)	Epilepsy (SEP)	Neck strain (SLA)
Asthma (SEP)	Eye problems (SLA)	Obesity (RF)
Back strain (SLA)	Gout (SLA)	Osteoporosis
Bleeding trait (SEP)	Hearing loss (SLA)	Phlebitis (MC)
Bronchitis, chronic (SEP)	Heart problems (SLA)	Rheumatoid arthritis (SLA)
Cancer (SEP)	High blood pressure (RF)	Stroke (MC)
Cirrhosis, liver (MC)	Hypoglycemia (SEP)	Thyroid problem (SEP)
Concussion (MC)	Hyperlipidemia (RF)	Ulcer (SEP)
Congenital defect (SEP)	Infectious mononucleosis (MC)	Other _____

11. Circle all medicine taken in last 6 months:

Blood thinner (MC)	Epilepsy medication (SEP)	Nitroglycerin (MC)
Diabetic pill (SEP)	Heart-rhythm medication (MC)	Estrogen
Digitalis (MC)	High-blood-pressure medication (MC)	Thyroid
Diuretic (MC)	Insulin (MC)	Corticosteroids
Asthma	Other _____	

12. Any of these health symptoms that occurs frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

0 = Never 1 = Practically never 2 = Infrequently 3 = Sometimes 4 = Fairly often 5 = Very often

a. Cough up blood (MC)
0 1 2 3 4 5

b. Abdominal pain (MC)
0 1 2 3 4 5

c. Low back pain (SLA)
0 1 2 3 4 5

d. Leg pain (MC)
0 1 2 3 4 5

e. Arm or shoulder pain (MC)
0 1 2 3 4 5

f. Chest pain (RF) (MC)
0 1 2 3 4 5

g. Swollen joints (MC)
0 1 2 3 4 5

h. Feel faint (MC)
0 1 2 3 4 5

i. Dizziness (MC)
0 1 2 3 4 5

j. Breathless with slight exertion (MC)
0 1 2 3 4 5

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Part 3. Health-related behavior

13. (RF) Do you now smoke? Yes No

14. If you are a smoker, indicate number smoked per day:

Cigarettes:	40 or more	20-39	10-19	1-9
Cigars or pipes only:	5 or more or any inhaled		Less than 5, none inhaled	

15. Weight now: _____lb. One year ago: _____lb..

16. Thinking about the things you do at work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

1. Much more active
2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

17. Now, thinking about the things you do outside of work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

1. Much more active
2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

18. Do you regularly engage in strenuous exercise or hard physical labor?

1. Yes (answer question # 19) 2. No (stop)

19. Do you exercise or labor at least three times a week?

1. Yes 2. No

Appendix 5: International Physical Activity Questionnaire (IPAQ)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

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5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

Appendix 6: Physical Activity Readiness Questionnaire

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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