

Collagen and Vitamin C Supplementation Increases Lower Limb Rate of Force Development

Dana M. Lis,¹ Matthew Jordan,² Timothy Lipuma,¹ Tayler Smith,¹ Karine Schaal,¹ and Keith Baar^{1,3}
¹Department of Neurobiology, Physiology & Behavior, University of California Davis, Davis, CA, USA; ²Canadian Sport Institute Calgary, Calgary, AB, Canada; ³Physiology and Membrane Biology, University of California Davis, Davis, CA, USA

Background: Exercise and vitamin C-enriched collagen supplementation increase collagen synthesis, potentially increasing matrix density, stiffness, and force transfer. **Purpose:** To determine whether vitamin C-enriched collagen (hydrolyzed collagen [HC] + C) supplementation improves rate of force development (RFD) alongside a strength training program. **Methods:** Using a double-blinded parallel design, over 3 weeks, healthy male athletes (n = 50, 18-25 years) were randomly assigned to the intervention (HC + C; 20 g HC + 50 mg vitamin C) or placebo (20 g maltodextrin). Supplements were ingested daily 60 min prior to training. Athletes completed the same targeted maximal muscle power training program. Maximal isometric squats, countermovement jumps, and squat jumps were performed on a force plate at the same time each testing day (baseline, Tests 1, 2, and 3) to measure RFD and maximal force development. Mixed-model analysis of variance compared performance variables across the study timeline, whereas t tests were used to compare the change between baseline and Test 3. **Results:** Over 3 weeks, maximal RFD in the HC + C group returned to baseline, whereas the placebo group remained depressed (p = .18). While both groups showed a decrease in RFD through Test 2, only the treatment group recovered RFD to baseline by Test 3 (p = .036). In the HC + C group, change in countermovement jumps eccentric deceleration impulse (p = .008) and eccentric deceleration RFD (p = .04) was improved. A strong trend was observed for lower limb stiffness assessed in the countermovement jumps (p = .08). No difference was observed in maximal force or squat jump parameters. **Conclusion:** The HC + C supplementation improved RFD in the squat and countermovement jump alongside training.

Keywords: performance, glycine, training, tendon, speed

Rate of force development (RFD) is highly correlated with sport performance that requires maximal mechanical power and speed (Lamas et al., 2012; Tillin et al., 2013) including weightlifting (Haff et al., 2005; Hornsby et al., 2017), jumps, throws (McLellan et al., 2011; Nuzzo et al., 2008), cycling (Stone et al., 2004), and sprinting (Slawinski et al., 2010). There are several determinants of RFD including muscle fiber type, neuromuscular activation, motor unit recruitment, and muscle-tendon unit stiffness (Buckthorpe & Roi, 2017). Various forms of training improve RFD in the general or untrained population. However, only high velocity and ballistic training have been shown to improve RFD in trained athletes (Lamas et al., 2012; Tillin et al., 2013). This type of training increases neural drive (motor unit recruitment and rate coding) and increases muscle-tendon unit stiffness (Earp et al., 2011).

Recent work suggests that collagen-based tissues (i.e., ligament, tendon, cartilage) are more dynamic than previously appreciated with similar turnover rates to skeletal muscle tissue (0.02%–0.13% per hour; Earp et al., 2011; Kalliokoski et al., 2007; Laurent, 1987; Miller et al., 2005, 2007; Smeets et al., 2019). Connective tissue adaptations, including increased stiffness arising from greater collagen content and cross-linking, decrease the risk of injuries since tendon stiffness is linearly related to failure strength (LaCroix et al., 2013; Marturano et al., 2013). Acute exercise is known to increase collagen synthesis (Langberg et al., 1999, 2001; Miller et al., 2007) as well as the expression of the primary enzyme involved in collagen cross-linking, lysyl oxidase (Heinemeier et al., 2009). The result is a

training-induced increase in connective tissue density and stiffness (Couppe et al., 2008; Kubo & Ikebukuro, 2019). While the relationship between exercise stimulus/loading and collagen synthesis is well established (LaCroix et al., 2013; Kubo & Ikebukuro, 2019), research on the role of dietary supplements that support these adaptations is in its infancy. Specifically, whether hydrolyzed collagen (HC) ingested prior to loading augments training-induced rates of collagen synthesis enough to measure changes in the capacity to rapidly generate muscle force (explosive strength) measured as the RFD is still theoretical.

In order to have a measurable effect on the mechanical function of connective tissues, the ingested amino acids must be digested, absorbed, transported to the target tissue, and then integrated into de novo collagen protein. Several studies have demonstrated that gelatin or an HC supplement ingested prior to exercise increased delivery of key amino acids (e.g., high amounts of glycine, proline, hydroxyproline) to connective tissue which may augment collagen synthesis in vivo (Konig et al., 2018; Oesser et al., 1999; Shaw et al., 2017). Amino acid levels peak in the blood about 40-60 min after ingestion (Alcock et al., 2019; Walrand et al., 2008). Microdialysate samples from the Achilles tendon in both young (21–30 years of age) and old-aged (60–75 years of age) participants demonstrated that amino acid levels peaked in the peritendinous space after 135 min for the younger age group and ~45 min for the older adults, following ingestion of a high proline, high glycine amino acid mix (Couture, 2020). In women with agerelated reductions in bone mineral density, 12 months of supplementation with 5 g of collagen peptides improved bone mineral density and demonstrated a favorable shift in bone markers, indicating increased bone formation and reduced bone degradation

Lis (dmlis@ucdavis.edu) is corresponding author, https://orcid.org/0000-0002-9759-7901.

(Konig et al., 2018). Finally, greater increases in procollagen Type I N-terminal propeptide (PINP; a marker of collagen synthesis in the blood) levels from baseline have been observed with gelatin and HC ingestion (Lis & Baar, 2019; Shaw et al., 2017). The effect of dietary collagen also demonstrates a dose–response pattern where 15 g of vitamin C-enriched gelatin significantly improved PINP, whereas 5 g was no better than placebo (PLA; Shaw et al., 2017). Overall, there is a developing line of research suggesting that the amino acids present in high amounts of HC and gelatin are well absorbed into the blood and transported to target tissues augmenting collagen synthesis and morphological changes (Oesser et al., 1999; Vieira et al., 2015).

To date, it is unknown whether these morphological changes in collagen-based tissues translate to improved mechanical muscle properties in athletes and whether pairing dietary collagen with training can improve adaptations more than exercise alone. The aim of this study was to determine whether supplementation with 20 g of vitamin C-enriched HC before heavy strength training, combined with a targeted maximal muscle power and RFD training program, improved explosive muscle performance including RFD in athletes more than a PLA.

Methods

Study Design

In a parallel design double-blind PLA-controlled manner, subjects were randomized to receive either the predetermined optimal vitamin C-enriched collagen dose based on PINP levels in the blood 4 hr after feeding (unpublished data; 20 g HC [GelitaTM; Calumet City, IL]+50 mg vitamin C; DSM Warrenville, IL) or maltodextrin (20 g; Tate & Lyle, Dayton, OH) PLA supplement. Supplements were weighed and prepackaged in an opaque food storage bag by an independent researcher not affiliated with the study. All supplements were packaged identically but labeled with a coding system to which all researchers were blinded. Subjects followed a prescribed progressive maximal power/RFD training program over 3 weeks alongside the heavy strength training program prescribed by their respective sport teams. After familiarization, exercise testing was conducted in the morning before training at baseline and once per week for the subsequent 3 weeks (Tests 1, 2, and 3) on the same weekday and at the same time each testing day (Figure 1).

Subjects

Based on previous work (Wilson et al., 1993), a power analysis calculation estimated that 23 subjects were required to achieve a statistical power of 80% for the primary outcome variable (isometric RFD). To allow for attrition, 25 subjects were recruited for each group (total n = 50). Healthy active collegiate athletes between the ages of 18 and 25 years were recruited from the University of California Davis football (n = 18) and rugby (n =19) teams and Reserve Officer Training Corps (ROTC) elite training group (n = 13). Inclusion criteria further included current full participation in sport, fewer than three musculoskeletal injuries in the previous 12 months, and no health or dietary restrictions that would be affected by the supplementation or study protocol. Females were excluded due to the confounding factor of alterations in collagen synthesis (the key framework of this study) throughout the menstrual cycle. Ethics approval was obtained through the Institutional Review Board at the University of California Davis and all subjects provided written informed consent to participate in this study. The trial was registered at clinicaltrials.gov (trial registration: NCT03293004).

Subjects were scheduled in team cohorts through the study period to ensure that the training content and training load were consistent, and the supplement was randomized within each cohort. Subjects followed their normal training program, prescribed by their sport and strength and conditioning coaches, along with a standardized and progressive maximal power/RFD training program prescribed by the researchers. This means that the training between cohorts was not controlled; however, by randomizing treatment within cohorts, any training differences between cohorts were accounted for. Subjects were familiarized with the study protocol, exercises, and testing procedures prior to baseline testing. During the baseline visit, anthropometric data including height (stadiometer) and mass (Tanita BF690W; Arlington Heights, IL) were collected. Subjects were familiarized with the exercise testing protocol and measurements were made to determine the bar height for the maximal isometric squat, standardized at 90% of the participant's high bar squat height position. Nutrition intake was recorded and verified by researchers the night before and each test day under the guidelines that stated subjects were to self-select food and fluids while consuming similar food the night and morning before testing. Subjects were required to abstain from alcohol throughout the study period. Habitual caffeine intake was permitted

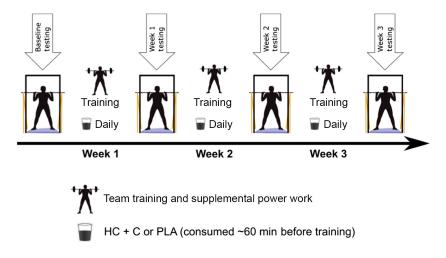


Figure 1 — Study schematic. HC+C = vitamin C-enriched hydrolyzed collagen; PLA = placebo.

and replicated prior to each test and no difference in caffeine intake was noted between groups.

Exercise Testing

Three maximal voluntary contractions of an isometric squat exercise (MIS), three repetitions of the countermovement jump (CMJ), and three repetitions of the squat jump (SJ), separated by a 30-s interrepetition rest interval and a 5-min interexercise rest interval, were performed on a force plate sampling at 1,000 Hz (Kistler, Novi, MI). Data were collected using Kistler MARS software (Kistler). Different Kistler force plates were used to test the vertical jumps and the MIS exercise. The raw data were then exported for analysis in a custombuilt software program (MATLAB version R 2019a; MathWorks, Natwick, MA). For the MIS, the predetermined bar height was set and fixed in place with ratchet straps tightened maximally to prevent any bar motion as described in previous work (Brady et al., 2020). Subjects were cued to "push up as hard and as fast as possible" and to hold the maximal voluntary contractions for 3 s. Participants were instructed to keep their hands firmly placed on the hips for the CMJ and SJ repetitions (Hebert-Losier & Beaven, 2014; Jimenez-Reyes et al., 2017; Samozino et al., 2008). The CMJ and SJ depth were self-selected by the participants. The SJs with small amplitude countermovements were discarded from the analysis.

Data Analysis

The MIS force–time curves were smoothed using the MATLAB smooth function with a 33 ms centered moving average window. A 100 ms average around the peak force value was calculated to obtain the maximum isometric force. The first derivative of the force–time curve was then calculated to obtain the maximum slope of the force–time curve. Using a 100-ms window, the average slope centered around the instant of maximum RFD was calculated to obtain the RFD.

The procedures for the CMJ and SJ analyses have been described in detail elsewhere (Caserotti et al., 2001; Jordan et al., 2015, 2018). Briefly, the velocity of the body center of mass (BCM) was obtained by time integration of the instantaneous acceleration signal calculated from the vertical ground reaction force ([Fz/body mass] – 9.81 m/s²). The CMJ eccentric deceleration and concentric movement phases were determined using the velocity of the BCM. The CMJ eccentric deceleration phase was defined as the time interval between the maximum downward negative velocity to the point of zero velocity achieved at the initiation of the ascent (deepest BCM position), whereas the CMJ concentric phase was defined from the starting point (zero BCM velocity) to the instant of jump takeoff. Mechanical muscle power exerted on BCM was derived continuously throughout the jumping movement by calculating the instantaneous product of Fz and BCM velocity. Peak mechanical muscle power was determined for both the CMJ and the SJ. Jump height was determined from the BCM vertical velocity at the instant of ground toe-off (jump height=takeoff velocity²/2 g). The CMJ eccentric deceleration phase and concentric phase net impulses were calculated by time integration of Fz over the respective time intervals along with the eccentric deceleration RFD obtained by average slope analysis of the force-time curve over the eccentric deceleration phase. Finally, SJ and CMJ contraction time (in seconds) were calculated, determined from the initiation of the vertical jump (start of descent) to the point of takeoff (toe-off) to obtain a modified reactive strength index (flight time to contraction time ratio – reactive strength index [RSI]). Outcome measures were normalized to body mass to allow betweengroup comparisons, and the maximum value obtained from each test was used for the statistical analysis.

Supplements

Supplements were prepared independent of the research group in powder form and precisely weighed to provide either 20 g powdered HC+50 mg powdered vitamin C or 20 g powdered maltodextrin. All supplements were packaged in opaque food safe bags and labeled with a blinded intervention code. Participants were instructed to mix the powder into 250 ml of water and to consume it as quickly as possible ~60 min before training. The supplement was taken every day of the study. A dosing calendar and text reminders were provided to ensure the supplement was taken once per day either prior to prescribed training or prior to team training if no prescribed training was assigned that day. On rest days, the supplement was taken with breakfast.

Prescribed Training

A maximal power and RFD training program (power training) was prescribed by the researchers and overlaid into each cohorts' planned team strength training. The power training was performed three times per week in a progressive loading manner over the study and included vertical drop jumps, vertical box jumps, and body weight loaded ballistic squats. Rugby and ROTC participants performed two sets of 10 repetitions for the first three training days after which the loading scheme progressed to three sets of 10 repetitions. On the third training day of each week, the number of sets was increased so that on the final training day subjects performed five sets of 10 repetitions. The training program progression was not possible for the football cohorts' program. Nevertheless, the training program included a progressive power training program with similar exercises including ballistic squats, plyometrics, and speed training in alignment with the training progressions outlined by the study researchers.

Statistical Analysis

A mixed-model analysis of variance was used to compare the effects of training and treatment on the performance measures. This included maximal RFD for the isometric squat. The CMJ outcome measures included the net concentric impulse, net eccentric deceleration impulse, the concentric and eccentric movement phase durations, and lower limb stiffness (leg spring stiffness). The vertical jump height, mechanical muscle power, and the modified RSI (flight time to contraction time ratio) were obtained for the CMJ and SJ. Differences were observed between the groups for some, but not all, parameters. A student's t test was used to compare the delta change from baseline to Test 3. A ROUT outlier test was performed to determine outlying data; however, outliers were not excluded from the analysis. Effect size was calculated using Cohen's d from t tests with <0.2 considered trivial, ≥ 0.2 to ≤ 0.5 considered small, ≥ 0.5 to <0.8 considered moderate, and ≤0.8 considered a large effect size. Data were analyzed using GraphPad Prism software (version 5.0; San Diego, CA). Data are presented as mean $\pm SD$ and the significance level was set at $\alpha < .05$ for all comparisons.

Results

Subjects

Fifty male subjects were recruited (age [mean \pm *SD*] = 18.8 \pm 2.0 years; height = 179.5 \pm 15.9 cm; weight = 85.8 \pm 18.3 kg; football, n = 18;

rugby, n = 12; ROTC, n = 18). Two subjects withdrew due to scheduling conflicts (n = 23 in vitamin C-enriched hydrolyzed collagen [HC+C] group and n = 25 in PLA group). Among the subjects that successfully completed the study, compliance with the study protocol and supplement consumption was 100%.

Maximal Isometric Squat Performance

There was a clear effect of training on maximal isometric squat force and RFD. Specifically, isometric force increased and RFD decreased over the course of the study for both groups (Figure 2). However, only the HC+C group demonstrated a subsequent recovery of RFD to the baseline value by Test 3 (p = .07; Figure 2b). As a result, the cumulative change from baseline to Test 3 showed that both groups increased maximum isometric force $(PLA = 7.09\% \pm 2.80\%; HC + C = 7.81\% \pm 2.60\%), whereas RFD$ decreased in the PLA group $(-16.20\% \pm 4.00\%)$ and was not different than zero in the HC+C group ($-2.13\% \pm 5.20\%$). When the change in RFD with training was determined (Figure 3), HC + C showed a significantly greater RFD than the PLA (p = .04) with a moderate effect size (Cohen's d = 0.5). One subject had an improvement of RFD of over 78.2% from baseline. If this data point were removed from analysis, significance for the HC+C compared with the PLA group is decreased without altering the effect size (p = .07; Cohen's d = 0.5).

Countermovement Jump Performance

There were no group differences in CMJ height (PLA = $3.18\% \pm 2.7\%$; HC+C= $2.4\% \pm 1.9\%$) over the study period (Figure 4a). Also illustrated in Figure 4, the PLA group decreased eccentric RFD (the capacity to reverse the downward acceleration of the BCM; Figure 4b) during training but with HC+C supplementation, eccentric RFD increased, resulting in an interaction between training and HC+C (p=.03). Although not significant, leg stiffness (Figure 4c; p=.14), concentric impulse (Figure 4e; p=.07), and RSI (Figure 4f; p=.3) tended to increase with training; HC+C supplementation did not alter the training effect. By contrast, maximal eccentric deceleration impulse decreased with training

in the PLA group, whereas the HC+C group increased eccentric deceleration impulse over the course of the study (Figure 4d; Training \times Time, p = .03). When the difference between baseline and Test 3 were determined, eccentric RFD was greater in the HC+C group compared with PLA group (Figure 5b; p=.04, Cohen's d = 0.6). However, a statistical outlier was found in the HC+C group. After reanalysis, the statistical difference between the HC+C and PLA condition was decreased (p = .07, Cohen's d = 0.8; Figure 5). Consistent with this finding, the HC+C group demonstrated a greater improvement in eccentric deceleration movement phase RFD compared to the PLA group (Figure 5c; p = .008, Cohen's d = 0.5). The changes in CMJ performance were likely due to the trend toward increased limb stiffness in the HC + C group (p = .081, Cohen's d = 0.5). Again, a single outlier was identified, and removal of this individual decreased statistical strength but not the effect size (p = .158, Cohen's d = 0.5). There were no differences found for mechanical muscle power, jump height, or the RSI.

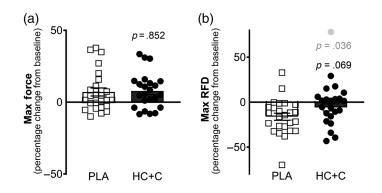


Figure 3 — Delta in the (a) max force is similar between groups in the maximal isometric squat. Delta in the (b) maximal RFD shows a significant improvement in the HC+C group compared with PLA. HC+C=vitamin C-enriched hydrolyzed collagen; PLA=placebo; RFD=rate of force development.

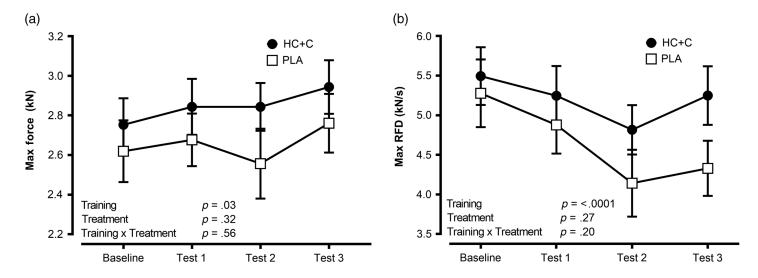


Figure 2 — In the (a) MIS, no difference was observed in maximal force over study timeline between groups. (b) Max RFD declined from baseline to Test 2 in both groups but improved in HC + C for Test 3. HC + C = vitamin C-enriched hydrolyzed collagen; PLA = placebo; MIS = maximal isometric squat; RFD = rate of force development.

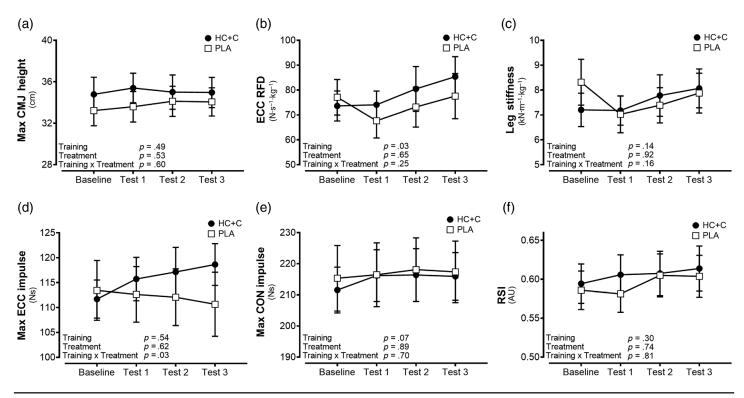


Figure 4 — The CMJ (a) jump height shows no difference between groups, (b) ECC RFD, (c) leg stiffness, (d) maximal ECC impulse shows a significant improvement over the study timeline, (e) maximal CON impulse, and (f) RSI show a non-significant trend toward greater improvement in the HC+C group compared to PLA. HC+C = vitamin C-enriched hydrolyzed collagen; PLA = placebo; ECC = eccentric; RFD = rate of force development; CMJ = countermovement jump; CON = concentric; RSI = reactive strength index.

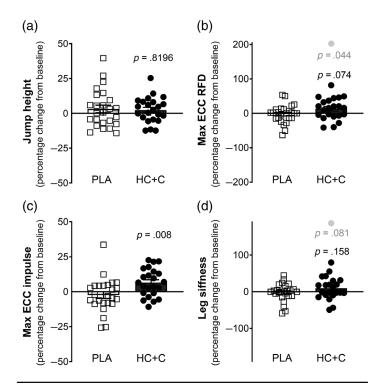


Figure 5 — Delta change from baseline to Test 3 is similar in both groups for (a) jump height. Significant improvement is shown in the delta for (b) maximal eccentric RFD, (c) maximal eccentric impulse, and (d) leg stiffness. HC+C=vitamin C-enriched hydrolyzed collagen; PLA=placebo; ECC=eccentric; RFD=rate of force development.

Squat Jump Performance

Consistent with the hypothesis that HC+C supplementation affects energy storage and return, there were no differences in SJ performance including jump height, mechanical muscle power, and RSI across the study (Figure 6).

Discussion

The present study demonstrated that consumption of 20 g of vitamin C-enriched collagen in conjunction with combined heavy strength and power training resulted in improved eccentric force capacity in the CMJ over a PLA-treated control. Notably, CMJ eccentric performance, including eccentric deceleration RFD and the net eccentric deceleration impulse, improved more in the HC+C group from baseline to Test 3.

The primary amino acids in dietary collagen supplements (HC, gelatin, and bone broth) may improve collagen synthesis in the body when paired with an appropriate loading stimulus (Lis & Baar, 2019; Shaw et al., 2017). To date, several studies have shown acute increases in PINP and longer term improvements in joint pain scores in athletes with 10–20 g of HC or gelatin prior to loading (Clark et al., 2008; Lis & Baar, 2019; Shaw et al., 2017). Although the current study did not measure PINP levels, the provision of the primary amino acids of collagen (glycine, proline, and the hydroxylated amino acids hydroxyproline and hydroxylysine) to the cells of the extracellular matrix and tendon may augment collagen synthesis. This suggestion is in direct contrast with milk proteins, such as casein that do not augment collagen synthesis following exercise (Moore et al., 2005; Trommelen et al., 2020). Interestingly,

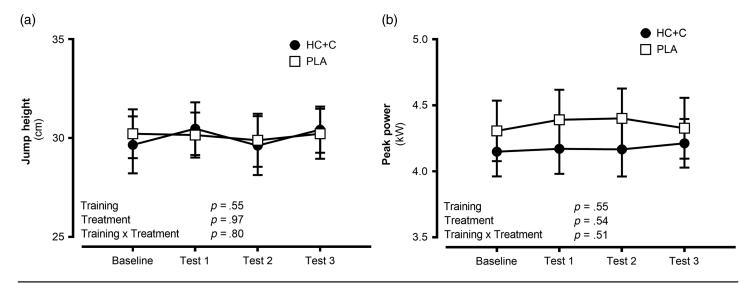


Figure 6 — No differences were observed over the study timeline between groups in the squat jump. HC+C=vitamin C-enriched hydrolyzed collagen; PLA=placebo.

following exercise and the consumption of 30 g of casein, there was a small decrease in glycine in the blood, suggesting that glycine may be a conditionally essential amino acid following a hypertrophy training stimulus such as resistance exercise (Trommelen et al., 2020). In contrast to milk proteins, dietary collagen is rich in glycine (>30% of all of the amino acid in the supplement) and this might be enough to augment collagen synthesis in response to training (Oikawa et al., 2020). Even though we did not directly measure collagen synthesis, combining the rapid delivery of glycine and other enriched amino acids (that peak 40–60 min postingestion; Alcock et al., 2019) with loading that is known to increase in collagen synthesis (Alcock et al., 2019; McAlindon et al., 2011; Paxton et al., 2012; Shaw et al., 2017) improved performance over exercise alone, providing indirect evidence that collagen supplementation increased matrix collagen synthesis.

Maximal isometric squat RFD, the primary outcome measure in the current study, declined over the study period for both groups; an enhancement of isometric RFD for the treatment group was not observed. However, isometric RFD is highly sensitive to increased training load and has been shown to decrease acutely consequent to neuromuscular fatigue and muscle damage (Penailillo et al., 2015; Thorlund et al., 2008). In the present study, only the treatment group demonstrated an enhanced recovery of isometric RFD back to baseline values by Test 3. It is unclear based on the present data whether or not HC + C supplementation elicited a protective effect against training-related neuromuscular fatigue and muscle damage. However, previous work shows collagen supplementation to have no beneficial effect on markers of muscle damage with eccentric training (Clifford et al., 2019). It is important to note that training specificity is a key factor contributing to changes in RFD (Blazevich et al., 2020). The training prescribed during the study was identical for the two groups and was probably not the reason for the decrease in RFD in the PLA group. Even though it is impossible to ensure that all participants lifted with the intention of generating maximal voluntary RFD, it seems unlikely that this could explain the group differences observed.

The observation of increased eccentric force capacity, including eccentric deceleration RFD in the CMJ, supports our hypothesis that training combined with HC + C supplementation enhanced explosive neuromuscular performance in athletes. Our working

hypothesis was that HC + C augmented collagen synthesis resulted in improved matrix mechanics, creating stiffer, more dense muscle ECM and tendons that transmit force faster, a key factor for explosive muscle performance (Maffiuletti et al., 2016; Wilkie, 1949). Support for this hypothesis comes from both acute and chronic changes in tendon and ligament structure and mechanics with training. Acutely, 4.5 and 8 hr after strength training, muscle collagen synthesis increased threefold (Moore et al., 2005). Additional support for the hypothesis that chronic training promotes positive connective tissue adaptations in athletes is seen in female soccer players who demonstrated an increase in ACL volume across an eight monthlong competitive season (Myrick et al., 2019). Even greater chronic adaptations to tendons are exemplified by elite fencers and badminton players who place significantly greater load on their lead leg during competition. Over time, this loading differential results in an increase in the cross-sectional area and the stiffness of the patellar tendon in the lead leg compared with the trial leg (Couppe et al., 2008). These adaptations likely result from a shift in collagen turnover, the balance between collagen synthesis, and degradation within the tissue. As with muscle hypertrophy, prolonged periods of positive protein balance result in a larger tissue that is mechanically stronger and more resistant to injury (Bayliss et al., 2016). The increase in eccentric force capacity, including RFD, observed in this study is suggestive of a shift toward positive collagen protein balance that is enhanced by dietary collagen consumption resulting in even greater adaptive changes to training.

Eccentric deceleration RFD and eccentric deceleration impulse reflect the capacity to reverse the downward acceleration of the BCM during fast stretch-shortening cycle movements and are important contributors to vertical jump performance (Barker et al., 2018; Krzyszkowski et al., 2020). Here, a more effective countermovement (unloading and eccentric deceleration phase) has been shown to amplify propulsive power in the vertical jump through an increase in the active state of the leg extensor muscles (Bobbert & Casius, 2005) and improved neuromechanical coupling of the eccentric–concentric movement phases (stretchshorten cycle; Cormie et al., 2010). The fact that the improvements in CMJ eccentric force capacity observed in this study occurred without an improvement in vertical jump height may have been attributable to the administration of the

combined heavy strength and power training protocol; there may be a divergent response in the relative improvement of explosive muscle performance versus jump height depending on the dose and sequencing of the training stimulus (Marshall et al., 2021). In further support of the notion that HC+C supplementation improved stretch-shorten cycle ability, we observed a trend toward increased lower limb stiffness or leg spring stiffness in the treatment group. However, a statistical difference between the HC+C group and PLA group was lost due to the removal of two participants who were deemed extreme responders and classified as statistical outliers. Nevertheless, the strong trend in support of an effect of HC+C supplementation on leg spring stiffness remains an important discussion point of our study. Stiffer tendons are better able to store and return elastic energy, minimizing the energetic cost of locomotion (Maffiuletti et al., 2016) and improving RFD. This is seen in jumping athletes where the plant leg has a significantly greater Achilles tendon stiffness compared with the drive leg (Kubo & Ikebukuro, 2019). Changes in joint stiffness with training have consistently translated to improved mechanical properties and performance in longer studies (Bayliss et al., 2016; Bojsen-Moller et al., 2005; Waugh et al., 2014). As with these older studies, the training protocol used in the current study tended to improve stiffness and explosive parameters in both groups, but larger improvements were consistently observed in the HC+C supplementation group. Support for the idea that the supplement was improving tissue stiffness and elastic energy storage comes from comparing the CMJ with the SJ. Even though statistical differences were seen in the CMJ, HC+C supplementation had no effect on any SJ parameters, likely because without the eccentric loading phase, the effect of better elastic energy storage was lost.

HC is a low quality protein with a digestible indispensable amino acid score of 0 due to the absence of tryptophan (Phillips, 2017). At rest and postexercise, older women who consume whey protein demonstrate significantly greater muscle protein synthesis compared with dietary collagen (Oikawa et al., 2020). This result would be expected as whey protein is a complete protein that is very high in leucine, an amino acid that triggers an increase in muscle protein synthesis (Breen & Churchward-Venne, 2012). By contrast, HC has very little to no leucine, and as a result leucine in the blood declines after feeding (Shaw et al., 2017). The target tissues, muscle compared with tendon, ligaments, and ECM similarly have very different amino acid make-up, suggesting that feeding different amino acids may result in targeted outcomes. Collagen proteins are highest in glycine which represents every third amino acid (Lis & Baar, 2019). As yet, no research has directly compared leucine rich whey protein with HC+C with collagen synthesis as the primary outcome measure. This study clearly needs to be done.

The current study was not without limitations. Although the study design favored ecological validity, the prescribed exercise was supervised by different strength and conditioning coaches for each sport cohort. This may introduce some inconsistency with the exercise prompts and execution of exercises led by the individual coaches. In addition, different sport cohorts and the inability to control diet over the study period increased variability in training type and load and the varying effects of nutrition on training adaptations. Performance outcomes in many sports are directly correlated with RFD. Results from the current study showed a strong trend and/or significantly better RFD with HC+C supplementation. The ability to produce force quickly during the initial phase of a voluntary contraction (0-300 ms), as reflected by a high RFD, is improved with explosive-type strength training (Maffiuletti et al., 2016). These data suggest that short-term dietary interventions designed to target connective tissues can augment velocity adaptations in athletes and may further the use of collagen supplementation beyond connective tissue injury and return to play (Baar, 2017).

Acknowledgments

This study was funded by the Gatorade Sports Science Institute, a division of PepsiCo, Inc. The views expressed in this manuscript are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc. D.M. Lis and M. Jordan refined the study design. PepsiCo's clinical operations personnel assisted with study tracking management. D.M. Lis organized, recruited, and executed study. K. Schaal, T. Lipuma, and T. Smith assisted with subject management and data collection. D.M. Lis, K. Baar, and M. Jordan completed the data analysis and interpretation. D.M. Lis, K. Baar, and M. Jordan wrote the manuscript. All authors approved the manuscript. The trial registration number was NCT03293004.

References

- Alcock, R.D., Shaw, G.C., Tee, N., & Burke, L.M. (2019). Plasma amino acid concentrations after the ingestion of dairy and collagen proteins, in healthy active males. *Frontiers in Nutrition*, *6*, 163. https://doi.org/10.3389/fnut.2019.00163
- Baar, K. (2017). Minimizing injury and maximizing return to play: Lessons from engineered ligaments. *Sports Medicine*, 47(Suppl. 1), 5–11. https://doi.org/10.1007/s40279-017-0719-x
- Barker, L.A., Harry, J.R., & Mercer, J.A. (2018). Relationships between countermovement jump ground reaction forces and jump height, reactive strength index, and jump time. *The Journal of Strength and Conditioning Research*, 32(1), 248–254. https://doi.org/10.1519/JSC.00000000000000160
- Bayliss, A.J., Weatherholt, A.M., Crandall, T.T., Farmer, D.L., McConnell, J.C., & Crossley, K.M., & Warden, S.J. (2016). Achilles tendon material properties are greater in the jump leg of jumping athletes. *Journal of Musculoskeletal & Neuronal Interactions*, 16(2), 105–112.
- Blazevich, A.J., Wilson, C.J., Alcaraz, P.E., & Rubio-Arias, J.A. (2020). Effects of resistance training movement pattern and velocity on isometric muscular rate of force development: A systematic review with meta-analysis and meta-regression. *Sports Medicine*, 50(5), 943–963. https://doi.org/10.1007/s40279-019-01239-x
- Bobbert, M.F., & Casius, L.J. (2005). Is the effect of a countermovement on jump height due to active state development? *Medicine & Science in Sports & Exercise*, *37*(3), 440–446. https://doi.org/10.1249/01. MSS.0000155389.34538.97
- Bojsen-Moller, J., Magnusson, S.P., Rasmussen, L.R., Kjaer, M., & Aagaard, P. (2005). Muscle performance during maximal isometric and dynamic contractions is influenced by the stiffness of the tendinous structures. *Journal of Applied Physiology*, *99*(3), 986–994. https://doi.org/10.1152/japplphysiol.01305.2004
- Brady, C.J., Harrison, A.J., & Comyns, T.M. (2020). A review of the reliability of biomechanical variables produced during the isometric mid-thigh pull and isometric squat and the reporting of normative data. *Sports Biomechanics*, 19(1), 1–25. https://doi.org/10.1080/14763141.2018.1452968
- Breen, L., & Churchward-Venne, T.A. (2012). Leucine: A nutrient "trigger" for muscle anabolism, but what more? *The Journal of Physiology*, 590(9), 2065–2066. https://doi.org/10.1113/jphysiol. 2012.230631
- Buckthorpe, M., & Roi, G.S. (2017). The time has come to incorporate a greater focus on rate of force development training in the sports injury rehabilitation process. *Muscles, Ligaments and Tendons Journal*, 7(3), 435–441. https://doi.org/10.11138/mltj/2017.7.3.435

- Caserotti, P., Aagaard, P., Simonsen, E.B., & Puggaard, L. (2001). Contraction-specific differences in maximal muscle power during stretch-shortening cycle movements in elderly males and females. *European Journal of Applied Physiology*, 84(3), 206–212. https://doi. org/10.1007/s004210170006
- Clark, K.L., Sebastianelli, W., Flechsenhar, K.R., Aukermann, D.F., Meza, F., & Millard, R.L., Deitch, J.R., Sherbondy, P.S., & Albert, A. (2008). 24-Week study on the use of collagen hydrolysate as a dietary supplement in athletes with activity-related joint pain. *Current Medical Research and Opinion*, 24(5), 1485–1496. https://doi.org/ 10.1185/030079908X291967
- Clifford, T., Ventress, M., Allerton, D.M., Stansfield, S., Tang, J.C.Y., Fraser, W.D., Vanhoecke, B., Prawitt, J., & Stevenson, E. (2019). The effects of collagen peptides on muscle damage, inflammation and bone turnover following exercise: A randomized, controlled trial. *Amino Acids*, 51(4), 691–704. https://doi.org/10.1007/s00726-019-02706-5
- Cormie, P., McGuigan, M.R., & Newton, R.U. (2010). Changes in the eccentric phase contribute to improved stretch-shorten cycle performance after training. *Medicine & Science in Sports & Exercise*, 42(9), 1731–1744. https://doi.org/10.1249/MSS.0b013e3181d392e8
- Couppe, C., Kongsgaard, M., Aagaard, P., Hansen, P., Bojsen-Moller, J., Kjaer, M., Magnussan, P. (2008). Habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon. *Journal of Applied Physiology*, 105(3), 805–810. https://doi.org/10. 1152/japplphysiol.90361.2008
- Couture, S. (2020). The effect of age on amino acid delivery to tendon. Kinesiology: Purdue University.
- Earp, J.E., Kraemer, W.J., Cormie, P., Volek, J.S., Maresh, C.M., Joseph, M., & Newton, R.U. (2011). Influence of muscle-tendon unit structure on rate of force development during the squat, countermovement, and drop jumps. *The Journal of Strength and Conditioning Research*, 25(2), 340–347. https://doi.org/10.1519/JSC.0b013e3182052d78
- Haff, G.G., Carlock, J.M., Hartman, M.J., Kilgore, J.L., Kawamori, N., & Jackson, J.R. (2005). Force–time curve characteristics of dynamic and isometric muscle actions of elite women olympic weightlifters. The Journal of Strength and Conditioning Research, 19(4), 741–748. https://doi.org/10.1519/R-15134.1
- Hebert-Losier, K., & Beaven, C.M. (2014). The MARS for squat, countermovement, and standing long jump performance analyses: Are measures reproducible? *Journal of Strength and Conditioning Research*, 28(7), 1849–1857. https://doi.org/10.1519/JSC.0000000000000343
- Heinemeier, K.M., Olesen, J.L., Haddad, F., Schjerling, P., Baldwin, K.M., & Kjaer, M. (2009). Effect of unloading followed by reloading on expression of collagen and related growth factors in rat tendon and muscle. *Journal of Applied Physiology*, 106(1), 178–186. https://doi.org/10.1152/japplphysiol.91092.2008
- Hornsby, W.G., Gentles, J.A., MacDonald, C.J., Mizuguchi, S., Ramsey, M.W., & Stone, M.H. (2017). Maximum strength, rate of force development, jump height, and peak power alterations in weightlifters across five months of training. *Sports*, *5*(4), 78. https://doi.org/10.3390/sports5040078\
- Jimenez-Reyes, P., Samozino, P., Pareja-Blanco, F., Conceicao, F., Cuadrado-Penafiel, V., & Gonzalez-Badillo, J.J., & Morin, J.-B. (2017). Validity of a simple method for measuring force-velocity-power profile in countermovement jump. *International Journal of Sports Physiology and Performance*, 12(1), 36–43. https://doi.org/10. 1123/ijspp.2015-0484
- Jordan, M.J., Aagaard, P., & Herzog, W. (2015). Lower limb asymmetry in mechanical muscle function: A comparison between ski racers with and without ACL reconstruction. *Scandinavian Journal of Medicine & Science in Sports*, 25(3), e301–e309. https://doi.org/10.1111/sms.12314

- Jordan, M.J., Aagaard, P., & Herzog, W. (2018). A comparison of lower limb stiffness and mechanical muscle function in ACL-reconstructed, elite, and adolescent alpine ski racers/ski cross athletes. *Journal of Sport and Health Science*, 7(4), 416–424. https://doi.org/10.1016/j. jshs.2018.09.006
- Kalliokoski, K.K., Bojsen-Moller, J., Seppanen, M., Johansson, J., Kjaer, M., Teras, M., & Magnusson S.P. (2007). Contraction-induced [18F]-fluoro-deoxy-glucose uptake can be measured in human calf muscle using high-resolution PET. *Clinical Physiology and Functional Imaging*, 27(4), 239–241. https://doi.org/10.1111/j.1475-097X.2007.00744.x
- Konig, D., Oesser, S., Scharla, S., Zdzieblik, D., & Gollhofer, A. (2018). Specific collagen peptides improve bone mineral density and bone markers in postmenopausal women-a randomized controlled study. *Nutrients*, 10(1), 97. https://doi.org/10.3390/nu10010097
- Krzyszkowski, J., Chowning, L.D., & Harry, J.R. (2020). Phase-specific predictors of countermovement jump performance that distinguish good from poor jumpers. *The Journal of Strength and Conditioning Research*, *34*(5). https://doi.org/10.1519/JSC.00000000000003645
- Kubo, K., & Ikebukuro, T. (2019). Changes in joint, muscle, and tendon stiffness following repeated hopping exercise. *Physiological Reports*, 7(19), Article e14237. https://doi.org/10.14814/phy2.14237
- LaCroix, A.S., Duenwald-Kuehl, S.E., Lakes, R.S., & Vanderby, R., Jr. (2013). Relationship between tendon stiffness and failure: A meta-analysis. *Journal of Applied Physiology*, 115(1), 43–51. https://doi.org/10.1152/japplphysiol.01449.2012
- Lamas, L., Ugrinowitsch, C., Rodacki, A., Pereira, G., Mattos, E.C., Kohn, A.F., & Valmor, T. (2012). Effects of strength and power training on neuromuscular adaptations and jumping movement pattern and performance. *The Journal of Strength and Conditioning Research*, 26(12), 3335–3344. https://doi.org/10.1519/JSC.0b013e 318248ad16
- Langberg, H., Rosendal, L., & Kjaer, M. (2001). Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans. *The Journal of Physiology*, *534*, 297–302. https://doi.org/10.1111/j.1469-7793.2001.00297.x
- Langberg, H., Skovgaard, D., Petersen, L.J., Bulow, J., & Kjaer, M. (1999). Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans. *The Journal of Physiology*, *521*, 299–306. https://doi.org/10.1111/j.1469-7793.1999.00299.x.
- Laurent, G.J. (1987). Dynamic state of collagen: Pathways of collagen degradation in vivo and their possible role in regulation of collagen mass. *American Journal of Physiology*, 252(1), C1–C9. https://doi. org/10.1152/ajpcell.1987.252.1.C1
- Lis, D.M., & Baar, K. (2019). Effects of different vitamin C-enriched collagen derivatives on collagen synthesis. *International Journal of Sport Nutrition and Exercise Metabolism*, 29(5), 526–531. https://doi.org/10.1123/ijsnem.2018-0385
- Maffiuletti, N.A., Aagaard, P., Blazevich, A.J., Folland, J., Tillin, N., & Duchateau, J. (2016). Rate of force development: Physiological and methodological considerations. *European Journal of Applied Physiology*, 116(6), 1091–1116. https://doi.org/10.1007/s00421-016-3346-6
- Marshall, J., Bishop, C., Turner, A., & Haff, G.G. (2021). Optimal training sequences to develop lower body force, velocity, power, and jump height: A systematic review with meta-analysis. *Sports Medicine*, *51*(6), 1245–1271. https://doi.org/10.1007/s40279-021-01430-z
- Marturano, J.E., Arena, J.D., Schiller, Z.A., Georgakoudi, I., & Kuo, C.K. (2013). Characterization of mechanical and biochemical properties of developing embryonic tendon. *Proceedings of the National Academy*

- of Sciences of the United States of America, 110(16), 6370–6375. https://doi.org/10.1073/pnas.1300135110
- McAlindon, T.E., Nuite, M., Krishnan, N., Ruthazer, R., Price, L.L., & Burstein, D. (2011). Change in knee osteoarthritis cartilage detected by delayed gadolinium enhanced magnetic resonance imaging following treatment with collagen hydrolysate: A pilot randomized controlled trial. *Osteoarthritis Cartilage*, 19(4), 399–405. https://doi.org/10.1016/j.joca.2011.01.001
- McLellan, C.P., Lovell, D.I., & Gass, G.C. (2011). The role of rate of force development on vertical jump performance. *The Journal of Strength and Conditioning Research*, 25(2), 379–385. https://doi.org/10.1519/JSC.0b013e3181be305c
- Miller, B.F., Hansen, M., Olesen, J.L., Schwarz, P., Babraj, J.A., Smith, K., Rennie, M.J., & Kjaer, M. (2007). Tendon collagen synthesis at rest and after exercise in women. *Journal of Applied Physiology*, 102(2), 541–546. https://doi.org/10.1152/japplphysiol.00797.2006
- Miller, B.F., Olesen, J.L., Hansen, M., Dossing, S., Crameri, R.M., Welling, R.J., Langberg H., Flyvbjerg A., Kjaer M., Babraj J.A., Smith K., & Rennie M.J. (2005). Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *The Journal of Physiology*, 567(3), 1021–1033. https://doi.org/10.1113/jphysiol.2005.093690
- Moore, D.R., Phillips, S.M., Babraj, J.A., Smith, K., & Rennie, M.J. (2005). Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions. American Journal of Physiology Endocrinology and Metabolism, 288(6), E1153–E1159. https://doi.org/10.1152/ajpendo.00387.2004.
- Myrick, K.M., Voss, A., Feinn, R.S., Martin, T., Mele, B.M., & Garbalosa, J.C. (2019). Effects of season long participation on ACL volume in female intercollegiate soccer athletes. *Journal of Experimental Orthopaedics*, *6*(1), 12. https://doi.org/10.1186/s40634-019-0182-8.
- Nuzzo, J.L., McBride, J.M., Cormie, P., & McCaulley, G.O. (2008).
 Relationship between countermovement jump performance and multijoint isometric and dynamic tests of strength. *The Journal of Strength and Conditioning Research*, 22(3), 699–707. https://doi.org/10.1519/JSC.0b013e31816d5eda
- Oesser, S., Adam, M., Babel, W., & Seifert, J. (1999). Oral administration of (14)C labeled gelatin hydrolysate leads to an accumulation of radioactivity in cartilage of mice (C57/BL). *The Journal of Nutrition*, *129*(10), 1891–1895. https://doi.org/10.1093/jn/129.10.1891
- Oikawa, S.Y., Kamal, M.J., Webb, E.K., McGlory, C., Baker, S.K., & Phillips, S.M. (2020). Whey protein but not collagen peptides stimulate acute and longer-term muscle protein synthesis with and without resistance exercise in healthy older women: A randomized controlled trial. *The American Journal of Clinical Nutrition*, 111(3), 708–718. https://doi.org/10.1093/ajcn/nqz332
- Paxton, J.Z., Hagerty, P., Andrick, J.J., & Baar, K. (2012). Optimizing an intermittent stretch paradigm using ERK1/2 phosphorylation results in increased collagen synthesis in engineered ligaments. *Tissue Engineering. Part A*, 18(3–4), 277–284. https://doi.org/10.1089/ten.tea.2011.0336
- Penailillo, L., Blazevich, A., Numazawa, H., & Nosaka, K. (2015). Rate of force development as a measure of muscle damage. *Scandinavian Journal of Medicine & Science in Sports*, 25(3), 417–427. https://doi.org/10.1111/sms.12241
- Phillips, S.M. (2017). Current concepts and unresolved questions in dietary protein requirements and supplements in adults. *Frontiers in Nutrition*, *4*, 13. https://doi.org/10.3389/fnut.2017.00013

- Samozino, P., Morin, J.B., Hintzy, F., & Belli, A. (2008). A simple method for measuring force, velocity and power output during squat jump. *Journal of Biomechanics*, 41(14), 2940–2945. https://doi.org/10.1016/j.jbiomech.2008.07.028
- Shaw, G., Lee-Barthel, A., Ross, M.L., Wang, B., & Baar, K. (2017).
 Vitamin C-enriched gelatin supplementation before intermittent activity augments collagen synthesis. *The American Journal of Clinical Nutrition*, 105(1), 136–143. https://doi.org/10.3945/ajcn. 116.138594
- Slawinski, J., Bonnefoy, A., Leveque, J.M., Ontanon, G., Riquet, A., & Dumas, R., Laurence, C. (2010). Kinematic and kinetic comparisons of elite and well-trained sprinters during sprint start. *The Journal of Strength and Conditioning Research*, 24(4), 896–905. https://doi.org/10.1519/JSC.0b013e3181ad3448
- Smeets, J.S.J., Horstman, A.M.H., Vles, G.F., Emans, P.J., Goessens, J.P.B., Gijsen, A.P., van Kranenburg J.M.X., & van Loon L.J.C. (2019). Protein synthesis rates of muscle, tendon, ligament, cartilage, and bone tissue in vivo in humans. *PLoS One*, 14(11), Article e0224745. https://doi.org/10.1371/journal.pone.0224745
- Stone, M.H., Sands, W.A., Carlock, J., Callan, S., Dickie, D., Daigle, K., Cotton, J., Smith, S.L., Hartman, M. (2004). The importance of isometric maximum strength and peak rate-of-force development in sprint cycling. *The Journal of Strength and Conditioning Research*, 18(4), 878–884.
- Thorlund, J.B., Michalsik, L.B., Madsen, K., & Aagaard, P. (2008).
 Acute fatigue-induced changes in muscle mechanical properties and neuromuscular activity in elite handball players following a handball match. *Scandinavian Journal of Medicine & Science in Sports*, 18(4), 462–472. https://doi.org/10.1111/j.1600-0838.2007.00710.x
- Tillin, N.A., Pain, M.T., & Folland, J. (2013). Explosive force production during isometric squats correlates with athletic performance in rugby union players. *Journal of Sports Sciences*, *31*(1), 66–76. https://doi.org/10.1080/02640414.2012.720704
- Trommelen, J., Holwerda, A.M., Senden, J.M., Goessens, J.P.B., van Kranenburg, J., & Gijsen, A.P., Verdijk, L.B., & Van Loon, L.J.C. (2020). Casein ingestion does not increase muscle connective tissue protein synthesis rates. *Medicine & Science in Sports & Exercise*, 52(9), 1983–1991. https://doi.org/10.1249/MSS.00000000000002337
- Vieira, C.P., De Oliveira, L.P., Da Re Guerra, F., Dos Santos De Almeida, M., Marcondes, M.C., & Pimentel, E.R. (2015). Glycine improves biochemical and biomechanical properties following inflammation of the achilles tendon. *The Anatomical Record*, 298(3), 538–545. https:// doi.org/10.1002/ar.23041
- Walrand, S., Chiotelli, E., Noirt, F., Mwewa, S., & Lassel, T. (2008). Consumption of a functional fermented milk containing collagen hydrolysate improves the concentration of collagen-specific amino acids in plasma. *Journal of Agricultural and Food Chemistry*, *56*(17), 7790–7795. https://doi.org/10.1021/jf800691f
- Waugh, C.M., Korff, T., Fath, F., & Blazevich, A.J. (2014). Effects of resistance training on tendon mechanical properties and rapid force production in prepubertal children. *Journal of Applied Physiology*, 117(3), 257–266. https://doi.org/10.1152/japplphysiol.00325.2014
- Wilkie, D.R. (1949). The relation between force and velocity in human muscle. *The Journal of Physiology*, 110(3–4), 249–280. https://doi.org/10.1113/jphysiol.1949.sp004437
- Wilson, G.J., Newton, R.U., Murphy, A.J., & Humphries, B.J. (1993). The optimal training load for the development of dynamic athletic performance. *Medicine & Science in Sports & Exercise*, 25(11), 1279–1286. https://doi.org/10.1249/00005768-199311000-00013