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# Hydrolyzed Collagen Supplementation on Lower Body Stiffness in Recreational Triathletes

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## Abstract

**Background:** Myotendinous stiffness is related to the collagen content of the muscle and tendon, and can be estimated during running by changes in vertical stiffness ($k_{vert}$) and the resulting modifications of the spatiotemporal parameters (on-off ground asymmetry and landing-takeoff asymmetry). Supplementation with amino acids found in collagen, such as proline, glycine, and hydroxyl proline, combined with ascorbic acid, improve collagen synthesis and potentially result in improved mechanical strength and stiffness.
**Objectives:** To determine if hydrolyzed collagen (HC) supplementation increases $k_{vert}$ and improves the spatiotemporal parameters during running in recreational triathletes.
**Methods:** Nine active males (weight; 68.4 ± 5.7 kg, height; 171.8 ± 5.4 cm, age; 32.5 ± 4.1 years; Vo2max; 53.15 ± 2.19 mL/kg/min) were randomly distributed into a collagen group (CollG, n = 5) and a control group (CG, n = 4). Participants were supplemented for 4 weeks with 15g HC (CollG) or 15g placebo (CG; maltodextrin), 3 times per week. One hour after supplementation, the participants of both groups were asked to perform four repetitions of short sprints to further stimulate collagen synthesis. The ground reaction forces were recorded during running at 4.44 m $s^{-1}$, 5.55 m.$s^{-1}$, and 6.66 m.$s^{-1}$ for assessment of $k_{vert}$ and the spatiotemporal step parameters.
**Results:** Both groups increased $k_{vert}$ with speed (4.44 - 6.66 m $s^{-1}$) from 24.8 ± 2.7 to 53.7 ± 16.5 N/m and from 25.1 to 49.8 N/m in the CollG and CG, respectively ($P < 0.0001$); however, there were no differences between groups before and after the supplementation period. As a consequence, the spatiotemporal parameters of running were also similar between groups.
**Conclusions:** Four weeks of HC supplementation does not improve the bouncing mechanism of running in recreational triathletes.

*Keywords:* Biomechanics, Collagen Supplementation, Spring-Mass System, Muscle-Tendon Efficiency, Elastic Bounce, Running

## 1. Background

The running stage in a triathlon competition has the greatest impact on the athlete's final ranking (1, 2). An important parameter affecting the ability to run more economically and, therefore, faster is myotendinous stiffness. Myotendinous stiffness is related to the collagen content of the muscle and tendon and can be estimated during running by changes in vertical stiffness ($k_{vert}$) of the lower body and by the spatiotemporal organization of the running step (3-7). $K_{vert}$ describes the vertical displacement of the center of mass (COM) in response to the vertical ground reaction force ($F_v$). An increased $k_{vert}$ has been related to greater neuromuscular adaptions and superior running performance (7-9), indicating that in runners with greater $k_{vert}$, the bounce is optimized, meaning a greater contribution of tendons relative to muscle within muscle-tendon units (10).

The spatiotemporal organization of the running step is characterized by two asymmetries of the rebound (3, 10): (1) the on-off ground asymmetry; and (2) the landing-takeoff asymmetry. Based on the vertical oscillation of the COM, the on-off ground asymmetry divides the step period into two parts: the effective contact time ($t_{ce}$) during which the $F_v$ is greater than body weight (BW), and the effective aerial time ($t_{ae}$) during which $F_v$ is lower than BW (11). At low speeds of running $t_{ce}$ is equal to $t_{ae}$, (symmetric rebound); meanwhile, at high speeds, tae is greater than $t_{ce}$ (asymmetric rebound) (11). On the other hand, the landing-takeoff asymmetry divides the $t_{ce}$ into two parts: the push time ($t_{push}$), period during which muscles perform posi-

tive work, and the brake time ($t_{brake}$), period during which muscles perform negative work. At low and intermediate running speeds, there is a difference between the duration of negative ($t_{brake}$) and positive work ($t_{push}$), where $t_{push} > t_{brake}$ (i.e. a landing-takeoff asymmetry) (4). The fact that $t_{push} > t_{brake}$ suggests an important contribution of the contractile machinery to the work production (5, 12, 13). However, at speeds above ~ 3.9 m.s$^{-1}$ $t_{push} \approx t_{brake}$, likely as a result of increased muscle activation and the substitution of the work contribution from the contractile machinery by the spring storage and recovery by tendons.

As speed increases, $F_v$ and $k_{vert}$ increase (14). The higher $k_{vert}$ results in an asymmetric on–off ground rebound, which has an antagonistic effect on the landing—takeoff asymmetry, as indicated by the enhanced spring-like mechanism of running (4). This spring function depends on the elastic properties of the musculoskeletal system, of which the structural proteins, titin, and collagen in the extracellular matrix (15), and the myotendinous junction (the connection between the muscle and tendon) play a prominent role in the resistance to elongation of the muscle (4), establishing the stiffness of the limb structure. It is relevant to consider that $k_{vert}$ is the resultant of the entire limb structure, like bone, muscle, tendons, and ligaments (14).

The function of the musculoskeletal tissues depends on the structure given by the collagen-rich extracellular matrix (ECM). The turnover of ECM is influenced by the physical activity and collagen synthesis, inducing an increase of mechanical load. This issue plays an important role in the force transmission in the muscle fiber (16). Previous studies have shown that supplementation with amino acids found in collagen, such as proline, glycine, and hydroxyl proline, combined with ascorbic acid, improve collagen synthesis (16-18), potentially resulting in improved mechanical strength and stiffness (19). However, to our knowledge, whether hydrolyzed collagen (HC) supplementation affects the mechanical stiffness of the lower body during running, in turn modifying the bouncing mechanism has not been assessed.

## 2. Objectives

Therefore, the aim of this study was to determine if HC supplementation modifies the bouncing mechanism of running. Based on the previously documented impact of HC supplementation on strength and muscle stiffness, we hypothesized that HC supplementation would result in increased $k_{vert}$ and an optimized rebound during running in triathletes, reflected by a smaller $t_{ce}/t_{ae}$ ratio and a greater $t_{brake}/t_{push}$ ratio in the CollG as compared to CG.

## 3. Methods

### 3.1. Participants

Nine active male triathletes were recruited (see Table 1 for individual characteristics of the CollG group and the CG group). Participants signed an informed consent form, and the study was approved by the Finis Terrae University Ethics Committee (resolution 36/2018) and performed according to the Helsinki declaration.

### 3.2. Study Design

In the first visit to the laboratory, maximum oxygen uptake (VO2max) was evaluated for each participant. The VO2max was determined by a breath-by-breath pulmonary gas exchange system (Ergocard, Medisoft, Belgium) during an incremental treadmill test. The starting speed was 8 km/h, with speed increments of 1 km/h every 60 s, without slope. A test was considered maximal when three of the following criteria were fulfilled: (1) VO2 plateau at peak exercise; (2) respiratory exchange ratio $\geq$ 1.10; (3) peak HR $\geq$ 90% of the theoretic maximal HR (220-age); and (4) indication of maximal exhaustion by the athlete. VO2max was defined as the highest 30 s average in oxygen uptake.

In the second visit to the laboratory (48 hours later), all athletes performed a 10-minute warm-up, including running on the treadmill at ~ 2.8 m.s$^{-1}$. After the warm-up, the participants ran on an instrumented treadmill (treadmill: Hp/Cosmos, Germany; force platform: Arsalis, Belgium) at three different speeds (3.9, 4.4 and 5.0 m.s$^{-1}$) for 30 seconds, with a 2-minute rest between each speed to avoid a possible fatigue effect. Laboratory room temperature was set to 23°C. Air humidity was in the 50 - 55% range. Hydration status was not recorded previous to testing, but participants were encouraged to maintain an adequate liquid intake a day previous to testing. All rocedures were performed at the same time of day.

### 3.3. Supplement Intake

The participants were then randomly assigned to either the collagen group (CollG, n = 5) or the control group (CG, n = 4); the athletes were supplemented 3 times per week for 4 weeks. One hour after consumption of their respective supplement, participants performed 4 repetitions of 100 m sprints on the track to increase the mechanical demand of the lower body, as exercise acutely stimulates collagen synthesis (Figure 1) (20). All beverages were delivered in a double-blind manner. The participants in the CollG received 15 g powder of HC (Great lakes gelatin, Grayslake, IL, USA, the content it's 36 mg of sodium, 12 g of collagen hydrolysate, and 43 kcal per 12 g serving). This was diluted in 500 mL of water. The participants in the CG received 15 g of maltodextrin (Chile Chemicals, Santiago, Chile) diluted in 500 mL of water.

**Table 1.** Individual Characteristics in Collagen Group (CollG) and Control Group (CG) [a]

| Variables | CollG (n 5) | CG (n 4) | 95% Confidence Interval for the Difference | | d (Cohen) |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| **Age (y)** | 33.6 ± 3.3 | 30 ± 4.1 | -0.1 | 7.3 | -1.03 |
| **Weight (kg)** | 66.4 ± 3.8 | 70.6 ± 6.1 | -7.8 | 0.4 | 0.85 |
| **Height (cm)** | 171.7 ± 6.7 | 172.5 ± 4.5 | -3.7 | 3.6 | 0.15 |
| **BMI ($kg.m^{-2}$)** | 22.5 ± 2.1 | 23.7 ± 1.6 | -4.8 | 2.5 | 0.63 |
| **VO2max ($L.min^{-1}$)** | 3.5 ± 0.2 | 3.7 ± 0.2 | -3.9 | 3.4 | 1.00 |
| **VO2max ($mL.kg^{-1}.min^{-1}$)** | 53.2 ± 1.9 | 53.2 ± 3.2 | -3.5 | 3.8 | 0.00 |
| **Training per week (h)** | 16.6 ± 2.1 | 17.3 ± 1.4 | -4.3 | 3.1 | 0.38 |

[a] Values are indicated as mean ± SD.

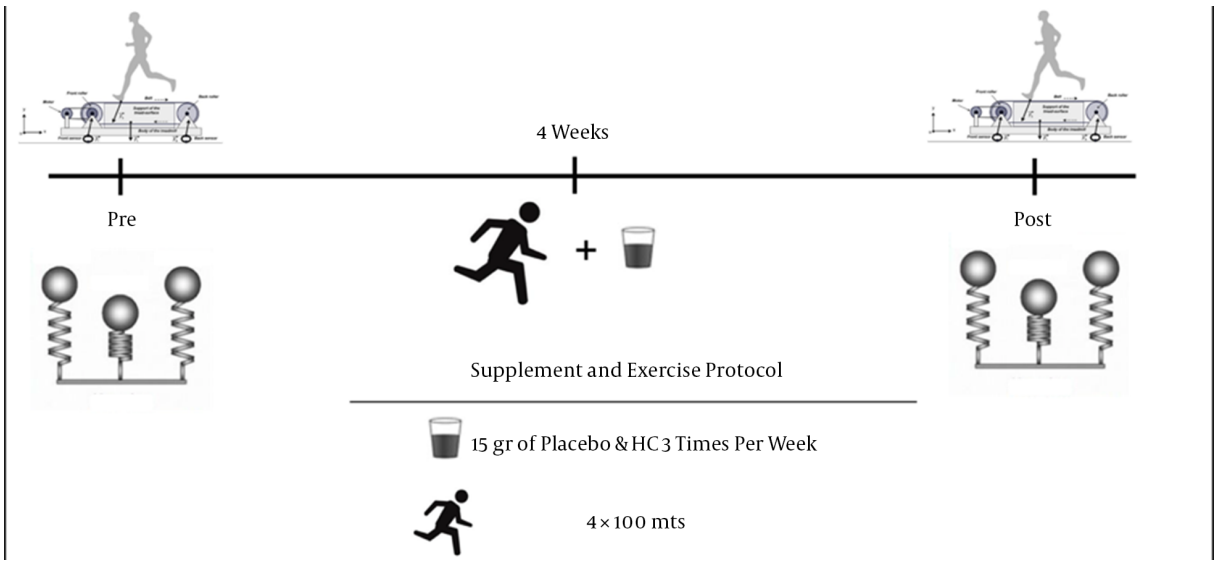


**Figure 1.** Study design (HC, hydrolyzed collagen).

### 3.4. Measurements of the Bouncing Mechanism of Running

Division of the steps. Steps were divided according to the $F_v$-time (Figure 2) The effective contact time ($t_{ce}$) was defined as the period during which $F_v \geq$ BW, and the effective aerial time ($t_{ae}$) was defined as the period during which $F_v <$ BW (11). The contact time ($t_c$) was defined as the period during which $F_v >$ 10 N, and the aerial phase ($t_a$) was defined as the period during which $F_v \leq$ 10 N. The work done to move the COM was computed from the ground reaction forces (GRF) using a similar method as described by Gosseye (21). The period $t_{push}$ and $t_{brake}$ were defined as the duration of the positive and of the negative work phases, respectively.

Vertical stiffness. At each step, the second peak [the "active" peaks, $P_{max}$, (22)] of $F_v$ was measured. The vertical displacement of the COM ($S_v$) was computed by double time integration of $F_v$/m, as described by Dewolf et al. (13). The maximum vertical displacement of $S_v$ during stance ($\Delta y$) was then measured. Finally, the vertical stiffness $k_{vert}$ was computed as $k_{vert} = P_{max}/\Delta y$, as in Butler, Crowell, & Davis (2003) (23).

### 3.5. Statistical Analysis

For each variable, the normality of the data was checked using the D'Agostino-Pearson test. Two-way ANOVAs and post-hoc Fisher's LSD corrections were performed to compare pre-and post-conditions for both groups. The variables considered were the speeds (4.44 $m.s^{-1}$, 5.55 $m.s^{-1}$, and 6.66 $m.s^{-1}$) and the groups CollG and CG. All statistical analysis was performed using Graph-

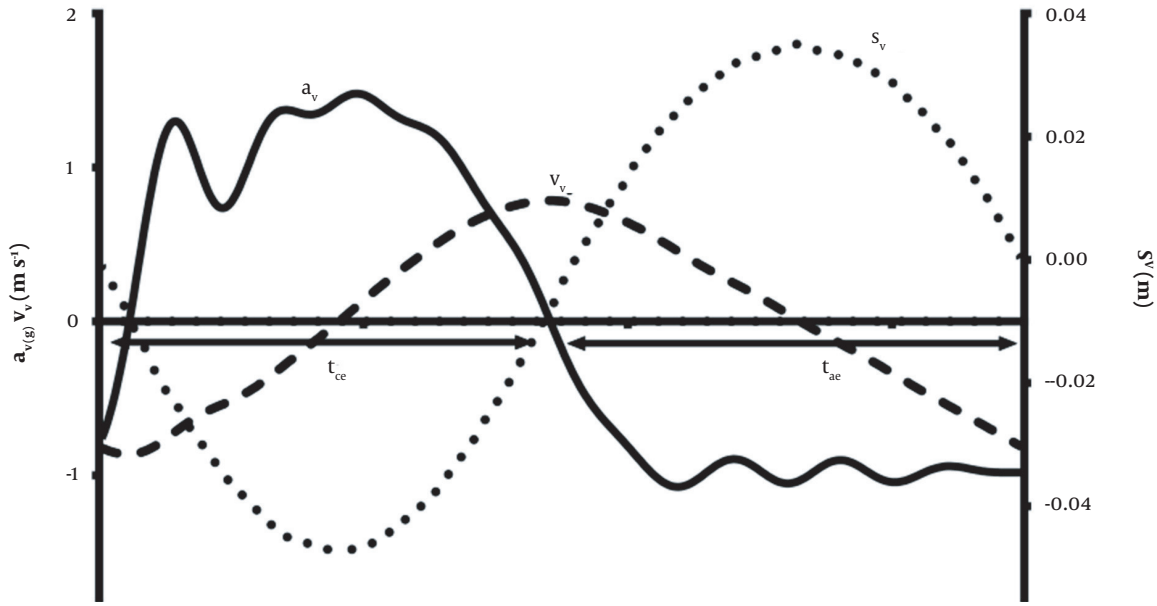


**Figure 2.** Representative figure for the vertical acceleration ($a_v$, solid line), vertical displacement of the center of mass ($S_v$, dotted line), vertical velocity of the center of mass ($V_v$, dashed line), effective contact time ($t_{ce}$, left arrow), effective aerial time ($t_{ae}$, right arrow) during running at 4.44 m.s$^{-1}$.

Pad Prism 6 software (USA). Alpha levels were set at 0.05. The effect size intragroup was determined by calculating Cohen's d values (Cohen, 1977). Cohen's d thresholds for small, moderate, and large effects were 0.2, 0.5, and 0.8, respectively. By intergroup post condition effect size, Hedges' g value was used (Hedges, 1981).

## 4. Results

### 4.1. Maximal Oxygen Uptake

The VO2max was not modified between the two groups and over time during the 4 weeks of supplementation (time P-value 0.377, group P-value 0.718, and interaction P-value 0.641) (Table 1).

### 4.2. Vertical Stiffness

In the CollG, $k_{vert}$ at 4.44 m.s$^{-1}$ speed was 26.7 ± 8.8 N/m and 24.7 ± 2.7 N/m pre- and post-condition, respectively (CI -3.2 to 7.19) (ES = 0.21). In the CG, $k_{vert}$ was 26.2 ± 1.8 N/m and 25.1 ± 1.8 N/m pre- and post-condition, respectively (CI -4.7 to 6.8) (ES = 0.43). At 5.55 m.s$^{-1}$ speed, $k_{vert}$ in the CollG was 27.4 ± 12.7 N/m and 32.8 ± 3.6 N/m (CI -15.1 to 4.4) pre-and post-condition, respectively (ES = -0.30). In the CG, $k_{vert}$ was 34.2 ± 1.8 N/m and 33.2 ± 3.7 N/m pre- and post-condition, respectively (CI -9.9 to 11.9) (ES = 0.40). At 6.66 m.s$^{-1}$ speed, $k_{vert}$ in the CollG was 41.56 ± 28.6 N/m and 53.7 ± 16.5 N/m pre- and post-condition, respectively (CI -26 to 1.6) (ES = 0.36). In the CG, $k_{vert}$ was 49.7 ± 9.4 N/m and 49.8 ± 9.4 N/m pre- and post-condition, respectively (CI-15.5 to 15.4) (ES = 0). The effect size post-condition intergroup was -0.17, -2.52, and 2.21 at 4.44, 5.55, and 6.66 m.s$^{-1}$ speeds, respectively.

No significant interaction between time and group was observed regarding $k_{vert}$ (Figure 3). Also, for $P_{max}$ there were no significant interaction (P-value 0.996), speed (P-value 0.807) and group effects (P-value 0.519) and no significant time effect (pre vs post; see Table 2) in either group. The $S_v$ was affected by the speed (P-value 0.002), but no significant interaction (P-value 0.0998), group effect (P-value 0.878), and time effect (pre vs post; see Table 2) were found.

### 4.3. The Asymmetries of the Rebound

No significant main effect or interaction were found between time and group regarding $t_{ce}$ and $t_{ae}$ (pre vs. post; see Table 3). However, as speed increased, $t_{ce}$ was affected in both groups (P-value < 0.0001) meaning that the rebound became more and more asymmetric ($t_{ce} < t_{ae}$). The ratio of $t_{ce}/t_{ae}$ did not change in the CollG (0.65 ± 0.05 in pre-condition and 0.64 ± 0.04 in post-condition; ES = -0.15), and the CG (0.64 ± 0.05 in pre-condition and 0.64 ± 0.04 in post-condition; ES = 0) (F = 0.05; P-value 0.981) (Figure 4).

### 4.4. Landing-Takeoff Asymmetry

No significant main effect of time and group and interaction were observed regarding $t_{push}$ and $t_{brake}$ (pre vs

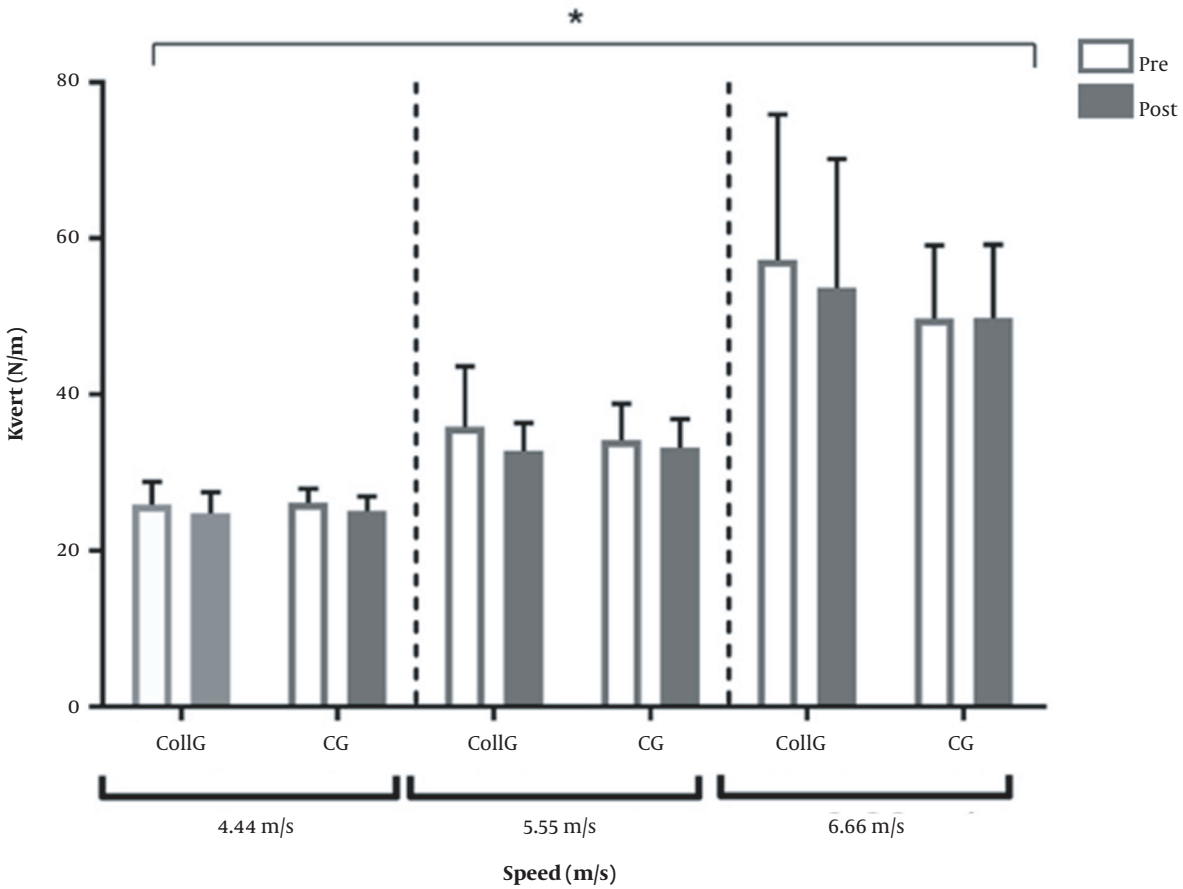


**Figure 3.** Mean and standard deviation of the vertical stiffness ($k_{vert}$) (*significant difference in function of increase speed).

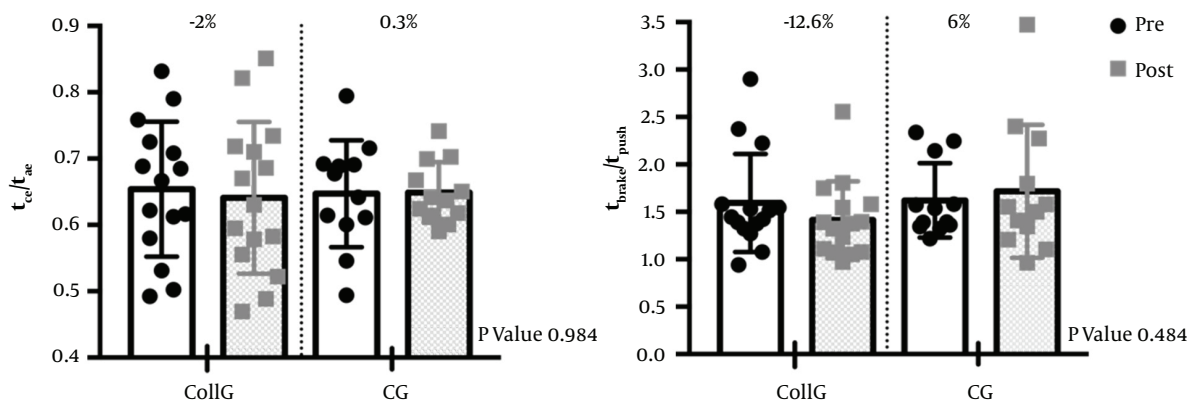


**Figure 4.** A, mean and standard deviation of the ratio $t_{ce}/t_{ae}$; B, of the ratio $t_{brake}/t_{push}$ of collagen group (CollG) and control group (CG).

**Table 3.** Average Range of the Effective Contact Time ($t_{ce}$) and the Effective Aerial Time ($t_{ae}$) During One Running Step in Collagen Group (Collg) and Control Group (CG) [a]

| Speed ($m.s^{-1}$) | $t_{ce}$ | | | | | | $t_{ae}$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre (s) | Post (s) | Change (%) | 95% Confidence Interval for the Difference | | d (Cohen) | Pre (s) | Post (s) | Change (%) | 95% Confidence Interval for the Difference | | d (Cohen) |
| | | | | Lower | Upper | | | | | Lower | Upper | |
| **4.44** | | | | | | | | | | | | |
| CollG (n 5) | 0.137 ± 0.01 | 0.136 ± 0.01 | -0.7 | -0.008 | 0.01 | 0.07 | 0.196 ± 0.02 | 0.201 ± 0.02 | 2.6 | -0.015 | 0.004 | 0.17 |
| CG (n 4) | 0.136 ± 0.01 | 0.142 ± 0.00 | 4.4 | -0.016 | 0.004 | 0.6 | 0.196 ± 0.00 | 0.203 ± 0.00 | 3.6 | -0.018 | 0.003 | 0.35 |
| **5.55** | | | | | | | | | | | | |
| CollG (n 5) | 0.12 ± 0.01 | 0.124 ± 0.01 | 3.3 | -0.013 | 0.004 | 0.28 | 0.187 ± 0.02 | 0.195 ± 0.02 | 4.3 | -0.018 | 0.001 | 0.28 |
| CG (n 4) | 0.119 ± 0.00 | 0.122 ± 0.00 | 2.5 | -0.012 | 0.007 | 0.03 | 0.186 ± 0.00 | 0.192 ± 0.00 | 3.2 | -0.017 | 0.004 | 0.05 |
| **6.66** | | | | | | | | | | | | |
| CollG (n 5) | 0.104 ± 0.01 | 0.105 ± 0.01 | 1.0 | -0.006 | 0.005 | 0.07 | 0.175 ± 0.02 | 0.181 ± 0.02 | 3.4 | -0.012 | 0 | 0.21 |
| CG (n 4) | 0.104 ± 0.01 | 0.106 ± 0.00 | 1.9 | -0.007 | 0.005 | 0.2 | 0.175 ± 0.00 | 0.173 ± 0.00 | -1.1 | -0.005 | 0.009 | -0.02 |

[a] Values are expressed mean ± SD in pre- and post-condition of CollG and CG.

**Table 2.** Average Range of the Maximum Vertical Displacement ($S_v$) and Average Second Peak ($P_{max}$) of Vertical Ground Reaction Force of the Center of Mass (COM) During One Running Step in Collagen Group (Collg) and Control Group (CG) [a]

| Speed ($m.s^{-1}$) | $S_v$ | | | | | | $P_{max}$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre (cm) | Post (cm) | Change (%) | 95% Confidence interval for the difference | | d (Cohen) | Pre (N/Bw) | Post (N/Bw) | Change (%) | 95% Confidence interval for the difference | | d (Cohen) |
| | | | | Lower | Upper | | | | | Lower | Upper | |
| **4.44** | | | | | | | | | | | | |
| CollG (n 5) | 8.1 ± 1.7 | 8.5 ± 1.7 | 4.9 | -1 | 0.2 | 0.16 | 2.8 ± 0.0 | 2.8 ± 0.3 | 2.7 | -0.2 | 0 | 0 |
| CG (n 4) | 8.1 ± 0.5 | 8.6 ± 0.3 | 6.7 | -1.2 | 0.1 | 0.85 | 2.7 ± 0.0 | 2.8 ± 0.1 | 4.4 | -0.2 | 0 | 1 |
| **5.55** | | | | | | | | | | | | |
| CollG (n 5) | 7.1 ± 1.6 | 7.6 ± 1.6 | 7.4 | -1.1 | 0.1 | 0.22 | 2.8 ± 0.4 | 2.8 ± 0.4 | 1.3 | -0.1 | 0 | 0 |
| CG (n 4) | 7.1 ± 0.3 | 7.5 ± 0.3 | 6.9 | -1.1 | 0.2 | 0.94 | 2.7 ± 0.0 | 2.8 ± 0.1 | 6.6 | -0.3 | 0 | 1 |
| **6.66** | | | | | | | | | | | | |
| CollG (n 5) | 5.9 ± 1.3 | 6.3 ± 1.6 | 6.5 | -0.7 | 0 | 0.19 | 2.7 ± 0.4 | 2.9 ± 0.4 | 3.5 | -0.4 | 0.1 | 0.35 |
| CG (n 4) | 5.9 ± 0.3 | 5.9 ± 0.3 | -0.3 | -0.4 | 0.4 | 0 | 2.7 ± 0.1 | 2.9 ± 0.2 | 5.8 | -0.4 | 0.1 | 0.89 |

[a] Values are expressed as mean ± SD in pre- and post-condition of CollG and CG.

post; see Table 4). However, as speed increased, $t_{brake}$ (P-value 0.0027) and to a greater extent $t_{push}$ (P-value 0.0004) were affected in both groups (P-value < 0.0027). As a result, at fast speeds, the difference between $t_{push}$ and $t_{brake}$ (the landing-takeoff asymmetry) was reduced. The ratio of $t_{break}/t_{push}$ had a non-significant decrease of 12.6% in CollG (1.59 ± 0.03 in pre-condition and 1.41 ± 0.08 in post-condition; ES = -2.10), meanwhile in the CG it had a non-significant increase of 6% (1.62 ± 0.04 in pre-condition and 1.71 ± 0.37 in post-condition; ES = -0.24) (F = 2.192; P-value 0.13) (Figure 4).

## 5. Discussion

The aim of this study was to determine if HC supplementation increased the bouncing mechanism of running in recreational triathletes. Our results show that four weeks of intermittent HC supplementation does not modify $k_{vert}$ nor the spatiotemporal parameters of the running step at speeds of 4.44, 5.55, and 6.66 $m.s^{-1}$ in recreational triathletes. However, a significant increase in $k_{vert}$ was observed as speed increased, leading to a greater on-off ground asymmetry ($t_{ce} < t_{ae}$) and a smaller landing takeoff asymmetry ($t_{push} \approx t_{brake}$), confirming the hypothetical link between vertical stiffness and the spatiotemporal organization of the steps proposed by Cavagna et al. (2009).

Even though there was no significant effect of the treatment in the CollG compared with the CG, it seems relevant to analyze the ES of the ratios $t_{brake}/t_{push}$ and $t_{ce}/t_{ae}$. The decrease of 12.6% (ES = 2.10) of $t_{brake}/t_{push}$ in the CollG reflects the possibility of a greater incorporation of tendons relative to muscle within muscle-tendon units compared to the CG (increase of 6%, ES = -0.24) (10). According to Cavagna et al. (12) we could speculate that during running, the CollG used a better rebound by the contribution between tendons and muscle, compared to the CG. However, da Rosa et al. (7) suggest that the asymmetry of $t_{brake}/t_{push}$ isn't sensitive enough to represent the bouncing mechanism of running and propose the ratio $t_{ce}/t_{ae}$ as a better measurement. We observed small ES in both groups $t_{ce}/t_{ae}$ without change in $k_{vert}$. Future studies should consider the use of the two asymmetries of the bouncing step to investigate the spring-mass behavior with the running performance, instead of other spatiotemporal parameters usually used in the literature (9).

We employed a protocol of four repetitions of 100-meter sprints after supplementation as a functional means to stimulate collagen synthesis over a period of four weeks, to induce an increase in lower limb stiffness without effect. In previous studies, collagen synthesis was increased in response to acute exercise (19, 20, 24, 25), e.g., Miller et

**Table 4.** Average Range of the Time of Push ($t_{push}$) and the Time of Brake ($t_{brake}$) During One Running Step in Collagen Group (CollG) and Control Group (CG) [a]

| Speed ($m.s^{-1}$) | $t_{push}$ | | | | | | $t_{brake}$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre (s) | Post (s) | Change (%) | 95% Confidence Interval for the Difference | | d (Cohen) | Pre (s) | Post (s) | Change (%) | 95% Confidence Interval for the Difference | | d (Cohen) |
| | | | | Lower | Upper | | | | | Lower | Upper | |
| **4.44** | | | | | | | | | | | | |
| CollG (n 5) | 0.72 ± 0.02 | 0.73 ± 0.01 | 1.4 | -22.9 | 21 | 0.44 | 0.1005 ± 0.01 | 0.09634 ± 0.01 | -4.1 | -14.3 | 22.7 | -0.31 |
| CG (n 4) | 0.66 ± 0.01 | 0.76 ± 0.01 | 15.2 | -34.6 | 14.4 | 7.07 | 0.1006 ± 0.01 | 0.09746 ± 0.01 | -3.1 | -17.5 | 23.8 | -0.24 |
| **5.55** | | | | | | | | | | | | |
| CollG (n 5) | 0.57 ± 0.01 | 0.64 ± 0.01 | 12.3 | -18.2 | 3.8 | 4.95 | 0.08982 ± 0.01 | 0.09037 ± 0.01 | 0.6 | -36.1 | 35.1 | 0.7 |
| CG (n 4) | 0.56 ± 0.01 | 0.61 ± 0.01 | 8.9 | -17.6 | 7 | 3.53 | 0.08722 ± 0.01 | 0.1247 ± 0.06 | 42.2 | -76.8 | 2.7 | 0.6 |
| **6.66** | | | | | | | | | | | | |
| CollG (n 5) | 0.5 ± 0.01 | 0.55 ± 0.01 | 10.0 | -11.1 | 2.4 | 3.53 | 0.07544 ± 0.00 | 0.07287 ± 0.00 | -3.4 | -4.6 | 9.7 | -0.27 |
| CG (n 4) | 0.46 ± 0.01 | 0.45 ± 0.01 | -2.2 | -6.9 | 8.3 | 0.7 | 0.07396 ± 0.00 | 0.07961 ± 0.01 | 7.6 | -13.7 | 2.4 | 0.51 |

[a] Values are expressed as mean ± SD in pre- and post-condition of CollG and CG.

al. (25) reported an increase of 0.077%.h$^{-1}$ of fractional synthetic rate (FSR) in tendon collagen after 1 h of one-legged kicking exercise at 67% of maximum workload. This suggests that collagen synthesis is sensitive to resistance exercise. Regarding endurance training, Langberg et al. (24) evidenced an increase of type I collagen in peritendinous tissue after 3 h of running (36 km). Further, there's evidence that supports that collagen synthesis is increased in response to prolonged training; specifically, Langberg et al. (26) reported an increased turnover of collagen type I in local connective tissue of the peritendinous Achilles' region after 4 and 11 weeks of physical training. However, it should be considered that this is the first study to date, which investigates the effect of exercise and supplementation of HC on lower limb stiffness.

With respect to the HC supplementation, Paxton et al. (19) reported an increase in collagen content, strength, and modulus of the sinew after treatment with ascorbic acid and proline (an amino acid found at a great amount in HC). Otherwise, Zdzieblik et al. (27) reported that supplementing a total of 1260 g of HC over 12 weeks combined with physical exercise (three times a week) increases muscle strength, fat-free mass, bone mass and decreases fat mass in elderly people. Also, Shaw et al. (18) declared that the supplementation of gelatine plus jumping road is effective in the increased collagen synthesis and modulus (stiffness) of the ligaments. It seems that the low volume, despite the high mechanical impact generated by sprint running used in our study, may not be sufficient to stimulate collagen synthesis and therefore induce an increase on stiffness. This means that the high impact of the stretch-shortening cycle (SSC) induced by jumping may be necessary to increase lower body stiffness as achieved in studies employing either a plyometric training program (28), or a muscular power exercise program (29).

We acknowledge that the current study has limitations. First, we did not perform anthropometric measurements on the participants. It is possible that changes in body composition could affect running mechanics; however, both groups had no changes in BW and responded similar to the supplementation protocol. Therefore, it is highly unlikely that this could affect the current findings. We also acknowledge that the present study has a small sample size, potentially leading to the null effects observed on lower-body stiffness. Future research should use a greater number of participants and associate the mechanical variables with body composition, especially skeletal muscle mass.

### 5.1. Conclusions

In conclusion, we showed that four weeks of 15 g of HC supplementation did not change $k_{vert}$ nor spatiotemporal organization of the running step in recreational triathletes. Future studies are still required to better understand the potential use of HC supplements for running performance enhancement.

## Footnotes

**Authors' Contribution:** Study concept and design, M. N., and H. Z.; Analysis and interpretation of data, A. D., M. N. and J. C.; Drafting of the manuscript, M. N., M. C., M. C.; Critical revision of the manuscript for important intellectual content, J. C., A. D., and H. Z.; Statistical analysis, M. N. and A. D.

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