THE EFFECT OF AGE ON AMINO ACID DELIVERY TO TENDON

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GLOSSARY

- *(Tendon) Adaptation:* A change to the tissue's structure and/or function in order to best suit its environment and the demands put upon it. This can include, but is not limited to, changes in tendon dimensions, structure (as evidenced by imaging techniques), mechanical properties, and/or blood flow[1].
- *(Amino Acid) Delivery:* The process by which orally-consumed amino acids are transported throughout the body.
- *Peritendinous Space:* The area around a given tendon. In this document, this term refers to the area surrounding the Achilles tendon unless otherwise specified.
- *(Amino Acid) Recovery:* The ratio of the concentration obtained in the microdialysis dialysate to the concentration in the extracellular, peritendinous fluid surround the catheter.
- *Resistance Exercise:* Exercise in which a muscle contraction is opposed by force to increase strength or endurance[2]
- *Reverse-Phase High-Performance Liquid Chromatography:* A process through which molecules are separated based on their hydrophobicity. A polar/hydrophilic mobile phase transports the microdialysis sample to the non-polar/hydrophobic column and here, various analytes in the sample are separated based on their different degrees of interaction with the non-polar column. Once separated based on polarity, ultraviolet light is used to determine the retention time of each substance.

ABSTRACT

As the soft tissue that transmits muscular forces to the bony skeleton, tendons play a key role in the human musculoskeletal system and must adapt over time to repeated mechanical loads to maintain functionality. Resistance exercise is one of the primary stimuli for increases in tendon size and strength in healthy, young individuals, but similar benefits are not observed in healthy, aged tendon. This failure in the elderly to adapt, along with the fact that tendons inevitably decline in morphology and function with age, puts older individuals at an increased risk of poor tendon health, subsequent injury, and a compromised quality of life. Alternative strategies to preserve and strengthen aged tendon has gone largely unexplored, highlighting a critical need to determine an effective stimulus for tendon adaptations in aging populations

The purpose of this study was to determine if age impacts the delivery of orally-consumed amino acids (AA) to the peritendinous Achilles space. If so, this investigation could serve as the foundation for future studies to evaluate the efficacy of supplemental amino acids for inducing positive adaptations in tendon during exercise. Furthermore, an enzyme-linked immunosorbent assay (ELISA) was performed to quantitively measure procollagen, a precursor of collagen, in the samples to evaluate the impact on supplemental amino acids on collagen synthesis.

To assess amino acid delivery, a microdialysis fiber was inserted into the peritendinous space anterior to the Achilles tendon in healthy young (n = 7, 21-30 years) and elderly (n = 6, 60-75 years) men and women after a twelve-hour fast. After baseline collection, subjects consumed a non-caloric, noncaffeinated AA beverage (16.65 g). Microdialysis samples were collected every fifteen minutes for four hours and analyzed using reverse-phase high-performance liquid chromatography.

Amino acid delivery to the peritendinous space was not compromised with age, and the administration of amino acids upregulated procollagen synthesis significantly more in healthy, elderly subjects than in those that are healthy and young. Though preliminary, these findings provide a strong foundation for future studies assessing the impact of amino acid supplementation as novel impetus for tendon adaptations in the elderly.

INTRODUCTION

Although understudied compared to skeletal muscle, tendons play a critical role in the human musculoskeletal system. Tendons, at their most basic structure, are soft tissues comprised of tropocollagen, a rope-like assembly of three alpha-helical amino acid chains. Tendon fibers are continuous with that of muscle, and the functions of the two structures are inherently related; without tendons, skeletal muscle forces could not act on the bony skeleton. In other words, tendons are essential to musculoskeletal function because they facilitate movement[3].

To maintain this ability to transfer force as we age, tendon must adapt over time to repeated mechanical loads. Resistance exercise is one of the primary stimuli for increases in tendon size and strength in healthy young subjects[4,5], but these adaptations are age-dependent; in similar studies evaluating the impact of resistance exercise (RE) in the elderly, healthy, aged tendon did not increase in size nor stiffness[6]. Because aged tendons do not adapt to resistance exercise[6], older individuals may be at an increased risk of poor tendon health and injury. This risk is exacerbated by age-related declines in tendon such as a reduced number of cells, unfavorable changes in cell morphology, reduced proteoglycan content, and a decrease in the length and number of cytoplasmic projections[7,8]. Approximately 50% of elderly individuals suffer from at least one painful tendinopathy that limits their ability to perform activities of daily living (ADL)[9] and over time, failure to perform ADL can lead to under-nutrition, dehydration, decreased mobility and independence, accelerated bone lose, delirium, depression, pressure ulcers, skin tears, and incontinence[10].

Collagen anabolism in tendon is largely understudied in the current literature, yet researchers have proposed that amino acid supplementation may improve the biochemical and biomechanical properties of these soft tissues. This theory is, in part, extrapolated from the fact that amino acid supplementation has consistently been shown in the literature to increase muscle protein synthesis. In addition, because procollagen is an amino acid chain, is it biologically plausible that supplementing these organic compounds could have a positive impact on tendon structure and function[11–13]. Amino acid supplementation has been shown to increase tendon cross-sectional area (CSA) and reduce subjective tendon discomfort in animal models, young adults and post-operative patients, but it is unclear if these findings would be observed in healthy elderly adults.

Statement of the Problem

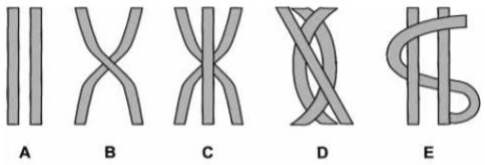
While several studies have been conducted to assess the effect of amino acid supplementation on tendon health and functionality[11–13], their findings cannot be translated to the current population of interest. The lack of applicability of these studies, in part, is that they do not consider the impact that aging may have on the delivery of orally-consumed amino acids. In other words, these findings cannot be generalized to elderly populations without confirming that age does not impair the delivery of an orally-consumed bolus of amino acids to the peritendinous space. The primary goal of this investigation was to determine if human aging impacts the delivery of amino acids to the peritendinous space following oral consumption. If a supplemental bolus of amino acids can augment the concentration of these organic compounds in the peritendinous space in healthy individuals ages 60-75, future studies can be conducted to determine their role in tendon remodeling. This study, therefore, is key to establishing the foundation for future research to improve musculoskeletal function in older individuals. The risk of tendon injuries increases with age, so this study may also have positive implications on the prevention and treatment of tendinopathies in aging populations. Based on literature that demonstrates that supplemental amino acids are successfully delivered to aged muscle[14], we hypothesize that aging will have a negligible (if any) effect on the overall delivery of amino acids to the peritendinous space following oral consumption. The secondary goal of this investigation was to measure the procollagen concentrations in each sample to assess if supplemental amino acids stimulate procollagen production, a marker of collagen synthesis.

LITERATURE REVIEW

Tendon Structure and Function

Tendon, the fibrous connective tissue that connects muscle to bone, is comprised of collagen and elastin within a proteoglycan-water matrix. Three alpha-amino acid procollagen chains associate to form a rope-like structure called tropocollagen (collagen fibrils), from which several tropocollagen chains cross-link in parallel to form a collagen fiber. Collagen fibers are the basic unit of tendon and are surrounded by endotenon, a connective sheath, to form a primary collagen bundle. Multiple primary bundles associate to form a secondary fiber bundle, secondary bundles cluster in parallel to form a tertiary fiber bundle, and ultimately, several tertiary fiber bundles are wrapped by the connective sheath epitenon to form tendon[15].

These collagen fibrils, which are composed of amino acid polypeptide chains, are oriented longitudinally, transversely, and horizontally within a given fiber. This arrangement enables the fibrils to cross-link longitudinally to form spirals and plaits with one another (Figure 1)[15]. Though collagen fiber crossing patterns vary greatly between tendons[16], each unique arrangement equips the tissue to handle rotational forces in these planes during everyday movement and physical activity. Furthermore, because these variable overlaps are likely to increase the tendon's tensile strength, the arrangement of these amino-acid derived collagen fibrils relates to the tendon's ability to transmit force during muscular contraction[15].



A = parallel running fibers, B = simply crossing fibers, C = crossing of two fibers with one straight-running fiber; D = a plait formation with three fibers, E = up-tying of parallel running fibers

Figure 1: Types of Collagen Fiber Crossing in Tendons (Kannus, 2000)

Each chain of procollagen contains the repeating amino acid motif Gly-X, Y, where X and Y can be any amino acid[17]. By nature of its presence in every third position, glycine makes up 33% of the amino acids in procollagen and thus, more broadly speaking, in tendon. Proline and hydroxyproline, which are typically (but not always) found in the X and Y positions, respectively, make up a combined 23%[17]. Just as procollagen plays a significant role in the structure of a given tendon, each of these amino acids in tropocollagen has a profound impact on the structure and function of tendon as a whole[18].

Glycine, proline, and hydroxyproline are all relatively small amino acids, which enables proper folding and tight winding of the triple alpha-helix in tropocollagen[18,19]. Furthermore, proline and hydroxyproline each contain an aromatic ring that contributes to helix binding[18]. Given the hierarchical assembly of tendon, proper formation of its smallest constituent is presumably critical. From a functional standpoint, glycine has been shown to modulate the inflammatory cascade in tendon by inhibiting pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 beta (IL-1β). Because these cytokines activate matrix metalloproteinases (MMPs) that degrade compounds in the extracellular matrix, inhibiting TNF-α and IL-1β may reduce both acute and chronic degradation of collagen molecules that contribute to poor tendon health[13]. In addition, proline enhances protein synthesis, regulates gene expression and cell differentiation, and is a precursor for hydroxyproline[18]. Once formed from proline, hydroxyproline facilitates the formation of water bridges within and between chains of collagen and enables tight packing of the triple helix[20]. This inherent importance of amino acids to collagen has led several researchers to theorize that supplemental amino acids may promote collagen fiber anabolism[11,13,21]. In other words, the increased concentrations of free amino acids in the peritendinous space may provide additional building blocks for tendon remodeling and adaptations.

Procollagen as a Marker of Collagen Synthesis

Collagen synthesis is a complex, multi-step process that begins with the transcription of DNA into messenger RNA (mRNA) within the nucleus of a fibroblast in connective tissue[22]. mRNA is then translated into a polypeptide chain in the endoplasmic reticulum, and following translation, this protein undergoes a series of enzyme modifications such as the hydroxylation of amino acids proline and lysine[22]. Three chains nucleate at the C-propeptide terminal to form a trimer, and this trimer is transformed from procollagen to tropocollagen once proteases cleave the N- and C-terminal propeptides[20] (Figure 2). Mature collagen, as mentioned above, then associates with other collagen molecules to form the fibrils, fibers, and fascicles of tendon. Because procollagen is a precursor to mature collagen, increased procollagen has been identified as an indicator of the upregulation of collagen synthesis[23]. In the context of the present study, an increase in procollagen may suggest that the tendon is adapting in response to amino acid supplementation.

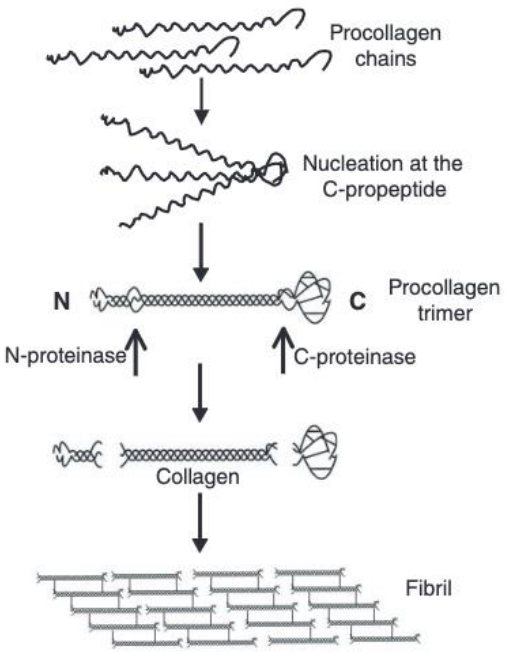


Figure 2: Overview of Collagen Synthesis (Canty and Kadler, 2005)

Tendon's Role in Maintaining Optimal Musculoskeletal Function

Tendon adaptations are essential for maintaining tendon health and optimal musculoskeletal function because, as previously mentioned, tendon functions as the intermediate between muscle and bone[15]. At one end of a tendon, its dense, fibrous connective tissue is continuous with that of muscle to form a musculotendinous junction (MTJ) Here, subsarcolemmal, transmembrane, and extracellular protein complexes link the muscle's basement membrane to the fibril-rich regions of the tendon[24]. Opposite of the MTJ is a tendon-to-bone insertion called an enthesis. Similar to the continuity of the muscle to tendon in the MTJ, a tendon connects to a bone's periosteum through a structurally continuous gradient[25]. Contractile forces from the muscle

are relayed through tendon to act on the bony skeleton and this, in turn, generates our everyday movement[15]. Without tendon acting as this intermediate, skeletal muscle would have no impact on musculoskeletal function. Because optimal musculoskeletal function is highly dependent on the role of tendon, these soft tissues must adapt over time to remain healthy and handle the repeated mechanical loads to which they are subjected.

Resistance exercise has been shown to increase tendon cross-sectional area (CSA) in young, healthy individuals[11], though the mechanism through which this occurs in unclear. Some researchers theorize that exercise stimulates an increase in blood flow and the release inflammatory and growth factors such as IL-1ß in both the tendon and peritendinous space[26]. IL-1ß, for instance, is then believed to upregulate the expression of Cyclooxygenase-2, MMPs,, and metalloproteinases with thrombospondin motifs (ADAMTS)[26,27]. These enzymes play a key role in regulating cell activity, matrix degradation, and influence fiber growth and development, and evidence shows that upon their release, collagen production in a tendon increases[26]. It is important to note, however, that further investigations into this theory are necessary because the overexpression of MMPs can lead to a degenerative, maladaptive response in tendon[1]. Regardless of the mechanism, findings by Kongsgaard et al., 2007 indicate that increases in collagen production ultimately lead to increases in the tendon's cross-sectional area in young subjects. In this study, twelve healthy young men completed twelve weeks of knee extension resistance training. One leg was designated a heavy resistance exercise protocol for the duration of the training (heavy-leg), while the other leg performed light resistance knee extensions (light leg). Though no significant effect was observed in the light leg, there were significant increases in the cross-sectional area of the patellar tendon's proximal and distal regions following the heavy resistance-exercise protocol[4]. Such increases in tendon CSA are beneficial because they reduce stress upon the tendon and increase its capacity to transmit force. Stress upon a tendon is defined as force transmitted divided by its CSA, so as the CSA increase, stress at the same force output is reduced. Because over-stressed tendons are susceptible to strain and further injury, these adaptations are imperative to preserve and potentially improve tendon health. Further, tendon CSA is proportional to tendon stiffness and thus its force transmission; as more fibers are added to tendon, its strength and stiffness increase and a tendon is able to transmit a greater amount of force at a given elongation[28].

In a study of sixteen highly-trained volleyball players and cyclists, tendon stiffness positively correlated to the rate of torque development (the speed at which muscular force can be generated and transferred via the tendon) as well as squat-jump height (the height a subject can jump immediately out of a bodyweight squat) (Figure 3)[29]. These findings suggest that increases in tendon CSA have positive implications on the musculoskeletal system's functional output, which is corroborated by the belief that tendon adaptations help a muscle operate as close as possible to its optimal length-tension ratio[30].

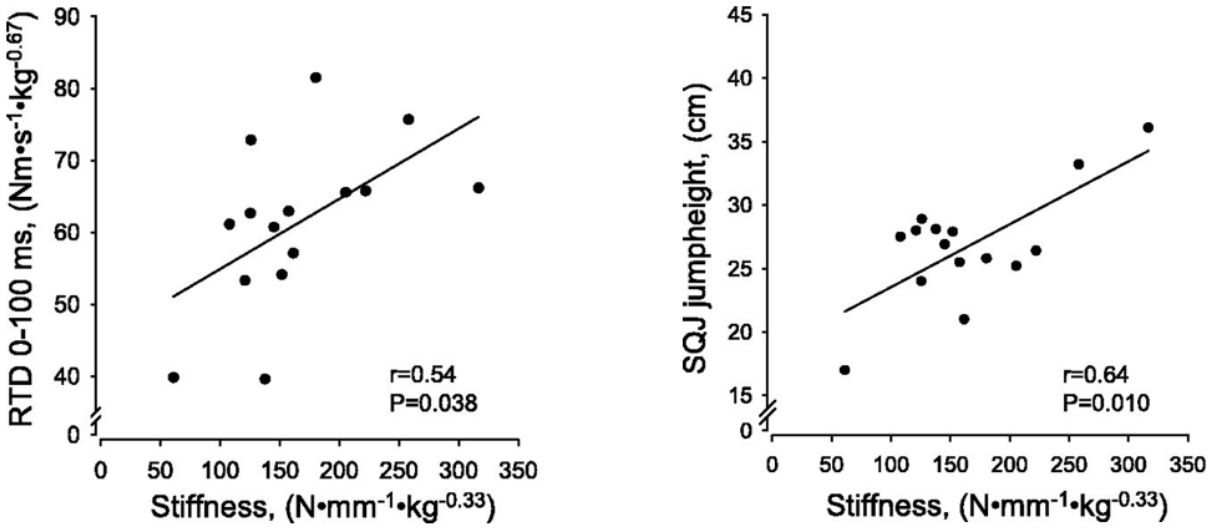


Figure 3: Analysis of the Vastus Lateralis Tendon-Aponeurosis Complex in 16 Trained Subjects (Bojsen-Møller et al. 2005)

Because aged tendons inevitably decline in morphology and function with age[31], resistance exercise-induced tendon adaptations would theoretically be very beneficial to older individuals. Tenocytes, the cells in tendon that produce collagen and elastin of the extracellular matrix, become less abundant and flatter with age[15], and studies have shown that older men have a reduced collagen concentration in their tendons compared to young men[32]. Furthermore, the proteoglycan content in aged tendon is also reduced compared to that of young tendon[33]. Because proteoglycans help facilitate the formation of tendon fibrils, aged tendon fibrils are often disorganized and therefore able to transmit less force[33]. The cytoplasmic projects that increase the contact between muscle and tendon also decrease in length and number with age, reducing the tendons force transmission and rendering them more susceptible to injury[33]. *In vivo* ultrasonography studies have confirmed these findings by demonstrating that tendon cross-sectional area, and thus stiffness, decrease with

age[27]. This reduction is at least in part because collagen turnover is reduced over time, leading to the deterioration of this primary component in tendon[30].

Unfortunately, the elderly population cannot counteract these declines with resistance exercise because tendon adaptations appear to be impaired in the elderly[6,34]. In a 2011 study assessing the impact of COX-inhibitors acetaminophen and ibuprofen on resistance training adaptations in twelve men and women ages 60-85, the control group performed a similar resistance exercise protocol to that in the study by Kongsgaard et al., 2007. After twelve weeks of knee extension resistance exercise training, there were no significant changes in patellar tendon CSA at the proximal, middle, or distal regions in these elderly subjects[6]. This suggests that collagen synthesis is not upregulated by exercise in an aged tendon. Furthermore, these elderly subjects exhibited no change in tendon stiffness, and thus the ability to transmit muscular forces, as a result of this RE protocol[6]. In a separate investigation evaluating the impact of aerobic cycle exercise training on quadricep muscle and patellar tendon hypertrophy, elderly women (70 +/- 2 years) demonstrated a significant increase ($P < 0.05$) in their quadricep muscle size but not their patellar tendon cross-sectional area after twelve weeks of training. Patellar tendon signal intensity, which may reflect changes in extracellular matrix, tissue hydration, collagen fiber structure, and/or the proteoglycan and glycosaminoglycan content of the tissue[35–38], also did not change after the training period (Figure 4). Taken together, these findings also suggest that aged tendon has an impaired adaptive response[39]. Other studies have demonstrated that elderly tendons can become stiffer in response to fourteen weeks of mechanical loading, yet these adaptations plateaued with continued exercise[34]. An aged tendon's impaired ability to adapt can reduce musculoskeletal function and this, along with the fact that tendon structure and function

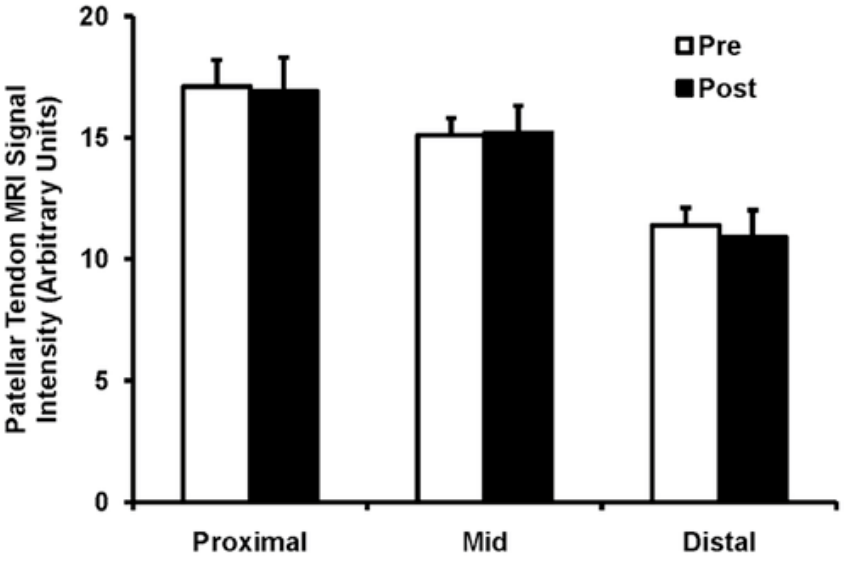


Figure 4: Patellar Tendon Signal Intensity Before and After Twelve Weeks of Aerobic Training in Older Women (Standley et al. 2003)

inevitably declines with age[31], may make elderly individuals especially susceptible to tendon complications.

Use of Amino Acid Supplementation in the Current Literature

Given that amino acids promote anabolism in skeletal muscle and other tissues, amino acid supplementation has been considered as an alternative method to promote collagen fiber anabolism. Pre-clinical animal studies and human clinical trials have assessed the impact of supplemental amino acids on systematically-induced tendinopathies[13], in conjunction with exercise protocols [11,21], and in those with pre-existing tendon injuries[12], yet to our knowledge, no studies have been conducted in healthy, elderly populations. The forthcoming studies reviewed may not be targeted at our population of interest, yet the findings nonetheless suggest that amino acid supplementation may successfully stimulate beneficial tendon adaptations.

Amino Acid Supplementation in Animal Models

Because researchers can more easily manipulate the lifestyle and diet of animal subjects, rodents are often used to evaluate the impact of amino acid supplementation on tendon health. In an investigation evaluating glycine's impact on the biochemical and biomechanical properties of the Achilles tendon following inflammation, researchers used collagenase to induce Achilles tendon inflammation in 60 male Wistar male rats[13]. These rats were then equally divided into five groups: the control (untreated, no inflammation), and four treatments: a standard diet for 8 days, a standard diet for 22 days, a 5% glycine diet for 8 days, or a 5% glycine diet for 22 days. In both the 8 and 22 day treatments, the 5% glycine diet upregulated de novo collagen synthesis and improved the organization of the extracellular matrix significantly more than the standard diet.

In addition, rats fed the 5% glycine diet for twenty-two days had hydroxyproline and glycosaminoglycans (GAGS) contents that were similar to those of the control group. Because hydroxyproline's aromatic ring contributes to the structure and strength of collagen[18], these findings suggest that glycine supplementation may improve tendon health. Glycosaminoglycans (GAGs), on the other hand, are a part of proteoglycan molecules that play a key role in tendon fibril formation and cell proliferation[40]. Because GAGs were also restored to control levels in the rats fed the 5% glycine diet, these results suggest that glycine supplementation may improve

tendon organization and promote a more rapid remodeling of the extracellular matrix following inflammation, respectively. Tendon birefringence also increased in glycine-fed rats, which is an indication of improved remodeling[13]. While a mechanism was not determined, an upward trend in matrix metalloproteinase-2 (MMP-2) activity was noted in the rats fed the 5% glycine diet. Given that MMP-2 plays an essential enzymatic role in ECM remodeling[26], it is plausible that the improvement in fibril arrangement can be attributed to glycine-induced upregulation of MMP-2 activity. Further investigation, however, is necessary because it is plausible that the upregulation of MMP-2 may also lead to increased soft tissue degradation.

These findings suggest that amino acid supplementation can improve the health of tendons in rats, yet they do not seamlessly translate to the elderly human population. Animals and humans have undoubtedly different physiologies, and this can impact the metabolism and utilization of amino acids. It is also important to note that this study used young opposed to elderly animal models. Because this study did not account for the effects of aging in rats, it is unclear if an older rat would have a similar response to a younger rat with the same tendon abnormalities. In addition, animal models also pose a challenge in the realm of musculoskeletal research because the biomechanics of animals versus humans are not the same. The manner in which a tendon is loaded on a day-to-day basis can influence its structural and mechanical properties[41], so an intervention of any sort in an animal model may not yield the same outcomes in human subjects. Lastly, the amino acids were used to treat systemically-induced tendinopathies in these animals, yet the present study intends to assess if orally-consumed amino acids can benefit aged but otherwise healthy tendon. Tendon abnormalities with varying etiologies may respond differently to a particular treatment, so it is unclear if the same conclusions could be drawn.

Amino Acid Supplementation in Conjunction with Exercise Protocols

Because eccentric resistance exercise is a commonly-prescribed conservative treatment for tendinopathies[42], most amino acid supplementation studies in humans investigate their ability to enhance exercise outcomes opposed to their efficacy as a standalone treatment. In a 2019 cross-over pilot study, twenty subjects with a pre-diagnosed mid-portion Achilles tendinopathy were allocated into one of two groups – Group AB or Group BA – and prescribed a six-month eccentric exercise program to be completed throughout the duration of the study[21]. Group AB consumed 2.5g of hydrolyzed specific collagen peptides (Tendoforte®) bi-daily for the first three months of the exercise protocol, and in months four through six, this supplement was replaced by a 2.5g maltodextrin placebo control. Group BA performed an identical exercise protocol with the order of their supplementation and placebo reversed. The Tendoforte® supplement was 22% glycine, but its exact composition was not disclosed for proprietary reasons.

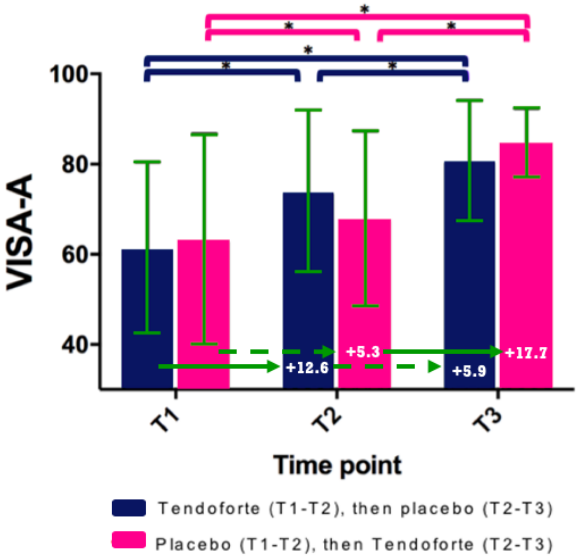


Figure 5: Victorian Institute of Sport Assessment - Achilles (VISA-A) Results Following Tendoforte Supplementation (Praet et al. 2019)

Victorian Institute of Sport Assessment – Achilles (VISA-A) Questionnaires were administered to all subjects to evaluate the severity of tendon symptoms and the impact that they had on the physical activity. During the treatment period that Group AB and Group BA received the glycine supplement (solid arrows), subjects reported a mean improvement of 12.6 and 17.7 points, respectively (Figure 5). When consuming the maltodextrin control (dashed arrows), scores improved by an average of 5.3 and 5.9 points in each respective group. The minimum clinically-important difference (MCID) for VISA-A assessments is 6.5 points, which means that both groups made clinically-significant improvements in tendon pain and functionality *only* while being supplemented with glycine. These improvements may be attributed to the supplementation, not the exercise protocol, because the latter was held constant throughout the duration of the study. This investigation was not adequately powered to detect statistical differences between the two intervention groups, yet the data suggest that the glycine supplement improved subjective pain and

functional limitations in the Achilles tendon. Further experimentation would be necessary to elucidate the significance of this trend.

These findings, albeit promising, must be considered with caution because the complete composition of the Tendoforte® supplement was not made available. The researchers assumed that glycine stimulated the perceived improvements in tendon structure and function, but it is possible that an undisclosed compound in the supplement was in fact the driving stimulus. Interpreting and applying these findings is also complicated because the subjects in this pilot study varied greatly from our population of interest. Our study targeted healthy, elderly individuals (60-75 years old) with no history of tendinopathies, yet the subjects studied by Praet et. al were on average 44 years old (44 +/- 8 years) and each had a diagnosed tendinopathy. Taken together, it is unclear if amino acid supplementation would stimulate tendon adaptations, as it appeared to in the work of Praet et al., in healthy, aged tendon.

Though amino acid supplementation is commonly coupled to eccentric resistance training in published studies, work by Farup et al. suggests that the oral consumption of amino acids enhances exercise adaptations during concentric protocols as well. In this investigation, twenty-two healthy, recreationally active men (average age of 23.9 years old) were allocated into either a whey protein hydrolysate or carbohydrate placebo supplementation group and completed 12 weeks (33 sessions) of maximal knee extensor training. Each leg of a given subject was randomly designated to perform either eccentric or concentric contractions for the duration of the study. After twelve weeks, the cross-sectional area of the subjects' patellar tendons was evaluated for exercise-induced growth. There were no changes in patellar tendon CSA in subjects ingesting the carbohydrate placebo, but significant increases were noted when subjects consumed the whey protein hydrolysate beverage. Of note, these increases were observed independent of exercise type; in subjects that consumed the whey protein hydrolysate beverage, tendon CSA area increased when performing both eccentric and concentric exercises. Though promising, these findings must be interpreted with caution. The subjects in the aforementioned study were three and a half to five decades younger than our target population, so it is unclear if orally-consumed amino acids would lead to tendon beneficial adaptations in our population of interest.

Amino Acid Supplementation in Injured, Aging Populations

To evaluate the role that supplemental amino acids play in human healing and recovery, Gumina et. al, 2012 randomly allocated eighty-seven post-operative patients to either a placebo control or treatment group. All patients had recently undergone a full-thickness large postero-superior rotator cuff repair surgery. In the treatment group, the subjects were supplemented with two sachets per day of arginine L-alpha-ketoglutarate, methylsulfonylmethane, hydrolyzed type I collagen and bromelain for three months, beginning on their first post-operative day. Because this supplement was given in the form of Tenosan®, a proprietary product, the concentrations of each compound are not publicly available. Control subjects received a placebo supplement for the same three-month duration.

Subjects in the treatment group had significantly higher integrity of their surgical repair after three months[12], as evidenced by a re-repture rates of 4.5% and 9.3% in the treatment and control groups, respectively. This suggests that the Tenosan® supplement improved the remodeling process and strength of the soft tissues comprising the rotator cuff following surgery, but these findings must be interpreted through a critical lens. It is possible that the observed improvements were a result of any of the four compounds in Tenosan®; for this reason, experimentation evaluating the effects of each compound in isolation would be necessary to draw more confident conclusions. Furthermore, the tendons of interest in this study were damaged and then surgically repaired, whereas many tendon complications in the elderly are a result of repeated and gradual overuse. Amino acid supplementation may have a significantly different impact on surgically repaired versus chronically-inflamed tendon, so one cannot assume that our elderly subjects, despite their similarity in age, would respond in a comparable fashion.

The Effect of Aging on Amino Acid Bioavailability

It has long been believed that nutrient bioavailability declines with age[43], but research evaluating the transport of orally-consumed amino acids in elderly individuals suggests otherwise. In an investigation of amino acid kinetics and muscle protein anabolism, both young (30 ± 2 years) and old (71 ± 2 years) healthy subjects were given supplemental amino acids in a post-absorptive state [14]. These subjects were untrained with no physical limitations, and the amino acid bolus (40g)

had a composition similar to that of meat proteins and was administered orally in a non-caloric, sugar-free beverage. Each of these conditions parallels our study protocol.

Muscle biopsies collected three hours after administration of the oral bolus demonstrated that amino acid delivery to the vastus lateralis increases to the same extent in both young and old healthy individuals[14]. These findings are corroborated by the discovery that advancing age has no independent effect on the secretion of gastric acids that play a key role in protein digestion [44]. These findings, among others, indicate that age-associated malabsorption is a consequence of disease, not aging itself[44,45].

Preliminary data collected by our research group suggests that is effective in detecting changes in the concentration of amino acids in the peritendinous space in young subjects following oral amino acid consumption. In this pilot study, microdialysis samples were collected from two young adults for six hours following a bout of acute exercise. These subjects consumed a bolus of amino acids ninety minutes into data collection. As shown in figure 3, the peritendinous levels (μM) of both leucine and glycine spiked after approximately sixty minutes. Future studies must be conducted to determine the statistical significance of these observations, as well as if they are also observed in elderly individuals, but this data gives us confidence in the efficacy of our proposed study design.

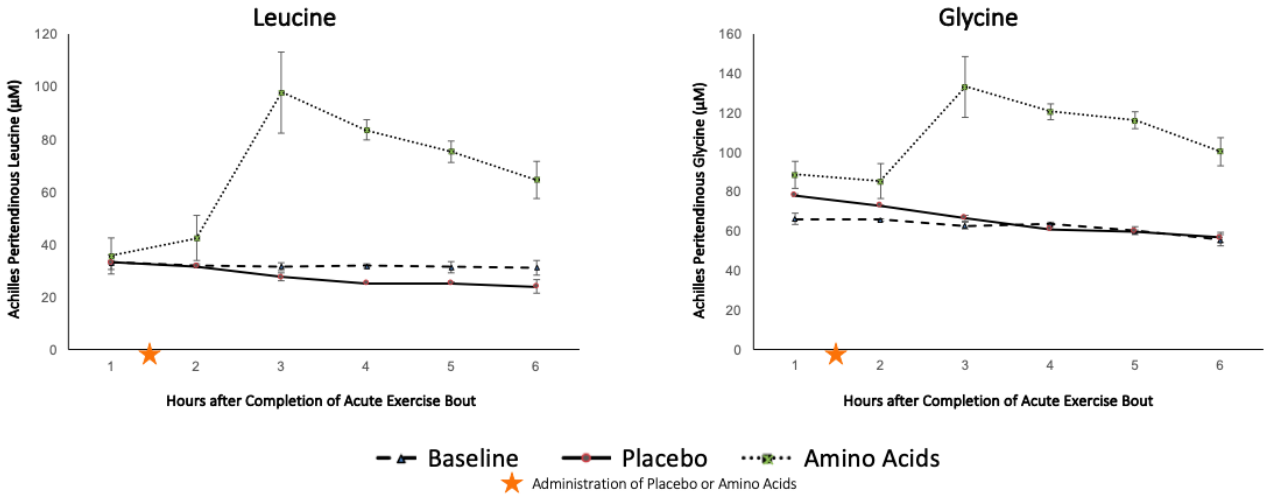


Figure 6: Peritendinous Amino Acid Concentrations - Preliminary Data from the Carroll Lab

Amino acids have been evaluated as a stimulus for tendon adaptations in multiple studies, yet the current literature has limited implications on healthy, elderly individuals. Natural aging puts elderly individuals at an increased risk of tendon degeneration, and this decline is further

exacerbated by their failure to adapt to resistance exercise. This population is at an increased risk of tendon complications yet has been largely overlooked. Easy-to-manipulate animal studies may shed light on the efficacy of amino acid supplementation, but animal physiology is undoubtedly different from that of humans and may impact the delivery of these compounds to the peritendinous space. Moreover, animal studies often evaluate systemically-induced inflammation, not that from natural aging, so it is even less unclear if the conclusions would apply. Investigations in young, healthy men provide a unique look into the potential impact of amino acid supplementation in humans, but the applicability of such studies is again unclear when considering the difference in age between these subjects and the elderly population of interest. With the possibility of using amino acid supplementation to support an anabolic response in tendons in human, elderly adults, we addressed this research gap by investigating if human aging alters amino acid delivery to tendon.

METHODS

Subjects

A total of thirteen men and women- seven young (aged: 21-30) and six elderly (aged: 60-75) – were recruited for this study. Potential subjects were excluded if their body mass index (BMI) exceeded 35 kg/m2, were on medications known to affect protein or collagen metabolism (e.g. acetaminophen, ibuprofen, or prescription cyclooxygenase inhibitors), had a previous history of tendinopathies or diabetes, or were sensitive to any of the ingredients in the study beverage. To ensure that a high activity level or fitness status did not confound results, all subjects also had to be either sedentary (one day or less per week of aerobic or resistance exercise for at least a year) or recreationally active (not training for competitive events). Before being enrolled in the study, subjects voluntarily gave informed written consent and were screened for any pertinent medical history. Subject characteristics can be found in Table 1. This study was approved by the Institutional Review Board of Purdue University, West Lafayette.

Table 1: Subject Characteristics

	Young (21-30 years)	Old (60-75 years)
Sex:		
Men	4	1
Women	3	5
Age ($\bar{x}$ ± SD)	26.6 ± 1.7	67.8 ± 3.8
BMI ($\bar{x}$ ± SD)	23.5 ± 2.9	24.3 ± 4.5

Overall Experimental Protocol

Because preliminary, unpublished work in our lab showed that amino acids are successfully delivered to the peritendinous space in young subjects, the seven young individuals in this study were used as controls for the elderly participants. On the day of testing, subjects arrived at Wastl Human Performance Laboratory following a 12-hour fast. The skin on the lateral and medial side of the participants' dominant ankle was prepared with an antiseptic (povidone-iodine), and then a local anesthetic (lidocaine 1%) was administered to both sides of the Achilles region. After confirming with the subject that the region was numb, a small needle was used to insert an ethylene oxide-sterilized microdialysis catheter in the peritendinous space anterior to their Achilles tendon.

Baseline samples were collected every fifteen minutes for one hour, and then subjects were instructed to consume the intervention beverage. This beverage was non-caloric, non-caffeinated and contained 16.65 grams of essential and non-essential amino acids. Following consumption, dialysate samples were collected every fifteen minutes to assess amino acid levels for four more hours. Reverse-phase high-performance liquid chromatography was used to measure the concentrations of metabolites in the peritendinous space at each collection timepoint.

Amino Acid (AA) Supplementation Protocol

The composition of this non-caloric, non-caffeinated beverage was adapted from a similar supplement shown to stimulate post-exercise skeletal muscle cell proliferation in older men[46]. Reidy et al.'s beverage contained 1.85 g leucine, but we chose to augment the leucine concentration to 3.5 g. This change was made in light of research demonstrating prolonged muscle protein synthesis in older men when given 3.5 g versus 1.85 g leucine post-exercise[47]. Furthermore, our research group decided to include non-essential amino acids - 2.0 grams glycine and 3.0 grams proline – in order to mimic the concentration of these amino acids found in 3.5 oz chicken and 4 oz beef, respectively. These amino acids were added to the beverage given their abundance in collagen, as well as to reflect what an individual might receive nutritionally from a standard diet. Each amino acid was weighed (± 0.02 g) and mixed into a standard 16 oz water bottle. Commercially-available Crystal Light flavoring was added to improve taste.

Table 2: Composition of Amino Acid Beverage

Amino Acid	Quantity (g)
Isoleucine	1.0
Phenylalanine	1.55
Valine	1.2
Leucine	3.5
Proline	3.0
Glycine	2.0
Histidine	1.1
Lysine	1.55
Methionine	0.30
Threonine	1.45
Total	*16.65 g*

Peritendinous Microdialysis

After a 12-hour fast, subjects arrived at Purdue University's Wastl Human Performance Laboratory. The subjects' skin was prepared with an antiseptic (povidone-iodine) and a local anesthetic (lidocaine 1%), and an ethylene oxide-sterilized microdialysis fiber was inserted into the peritendinous space anterior to the Achilles tendon. Insertion of this linear, flow-through

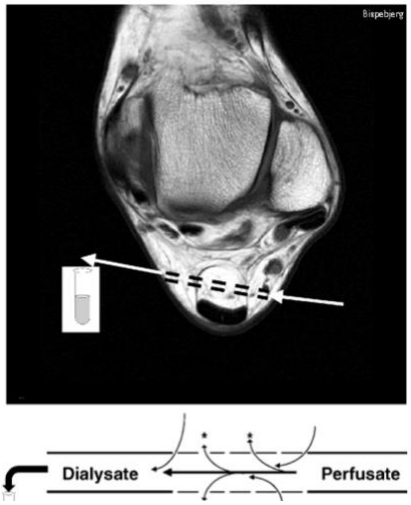


Figure 7: Schematic of the Microdialysis Fiber Insertion (Magnusson et al. 2003)

catheter established a system in which perfusate could be pumped at a constant rate into the catheter, travel under the skin, and be collected as dialysate in a 0.6 mL conical tube following the exchange of solutes in the peritendinous space. A schematic has been adapted from Magnusson et al.[48] to illustrate this process (Figure 7).

A saline solution containing sarcosine was perfused through the catheter at a flow rate of 2 μL/min. To collect and store the dialysate for future analysis, the tip of the catheter tubing was threaded into a 0.6 mL conical tube that was gently adhered to the medial side of the participants' ankle. After sixty minutes of baseline collection, participants consumed a mixed amino acid beverage (as described in the 'Amino Acid Supplementation Protocol'). Dialysate samples were then collected every fifteen minutes to obtain a time-dependent curve. After the data collection, the fiber was carefully removed, the skin was cleaned, and a band-aid was placed over the insertion site on both the lateral and medial side. All participants were contacted six hours post-testing to monitor the catheter insertion site and no adverse events were reported.

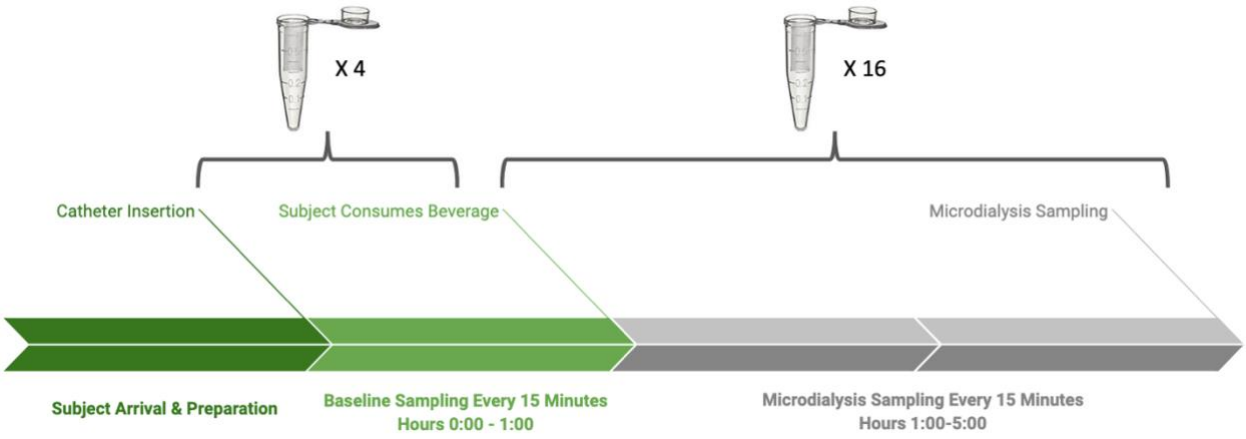


Figure 8: Timeline of Microdialysis Protocol

Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)

Plasma amino acid (AA) concentrations, and thus the delivery of AA to the peritendinous space, were measured using reverse-phase high-performance liquid chromatography (RP-HPLC). Before performing RP-HPLC, two mobile phase solutions were prepared: mobile phase A, which consisted on 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, pH 8.2, and 5 mM NaN_2, and mobile phase B, a 45:45:10 ratio of HPLC-grade acetonitrile, methanol, and water. A 0.1 M HCl standard solution was then produced to create amino acid standards of 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 µM, and 100,000 µM solutions for each amino acid were prepared using the Sigma Amino Acid Standard (AAS18). Standards and samples were then deprotonated with 10% trichloroacetic acid and derivatized using 0.5M Borate buffer, o-phthaladehyde (OPA, Agilent 5061-3335), fluroenylmethyloxycarbonyl chloride (FMOC, Agilent 5061-3337)[49]. Derivatized standards and samples were injected onto an Agilent 1100 series high-performance liquid chromatography system. Of note, the derivatized samples were combined in the following manner to obtain a large enough volume necessary for analysis: samples 3-4, 5-6, 7-8, 9-10, 11-12, 13-14, 15-16, 17-18, and 19-20. The timepoint of each new, pooled sample was defined as the average of the times of the two samples that were combined. Separation of the AAs was achieved using Agilent Eclipse Plus C18 4.6x100 mm, 3.5µm column with a Restek Ultra C18 Guard Column (10 x 4.0 mm). Peaks were monitored at 230 nm excitation/450 nm emission (G1321A; Agilent Technologies, Santa Clara, CA). The concentration of individual AAs was determined by comparison with a standard curve developed from known AA concentrations for each AA.

Statistical Considerations

The entire data set could not be processed due to the unforeseen circumstances of the COVID-19 pandemic, so the forthcoming results and conclusions will be based off of raw data. In the future, full statistics will be run following completion of the ELISA and RP-HPLC on all subjects using a two-way analysis of variance (ANOVA) with time and age evaluated as factors. Amino acid concentrations at each time point will be compared to concentrations measured during the baseline sampling, and the area under the curve for each amino acid present will also calculated.

RESULTS

Amino acid delivery to the Achilles peritendinous space was not impaired with age when comparing young versus old subjects. In fact, preliminary results suggest that amino acid delivery is enhanced in elderly subjects. This variability, however, may decrease with a larger sample size and after the probe recovery adjustment. Basal procollagen concentrations in the peritendinous space were significantly higher in old versus young subjects. Amino acid concentrations in the peritendinous space did not correlate with procollagen production one-hour after amino acid supplementation in young or old subjects, but there was a weak positive correlation two- and three-hours post-consumption in young subjects.

Amino Acid Delivery to the Peritendinous Space

As hypothesized, total amino acid delivery to the peritendinous Achilles space was unaffected by age (Figure 7). These results initially suggest that amino acid delivery was *enhanced* in the elderly subjects, but this variation between young and old subjects is likely to decrease once the sample size increases and the probe recovery adjustment is complete. It is important to consider that two amino acids – proline and sarcosine- were not included in this assessment. Unlike the other primary amino acids, proline is a secondary amino acid and therefore requires a different detection setting in order to be assessed. Running a separate assay was in the original methodology, but time constraints made this unfeasible. In addition, sarcosine was present in the peritendinous space but not included in the data set presented

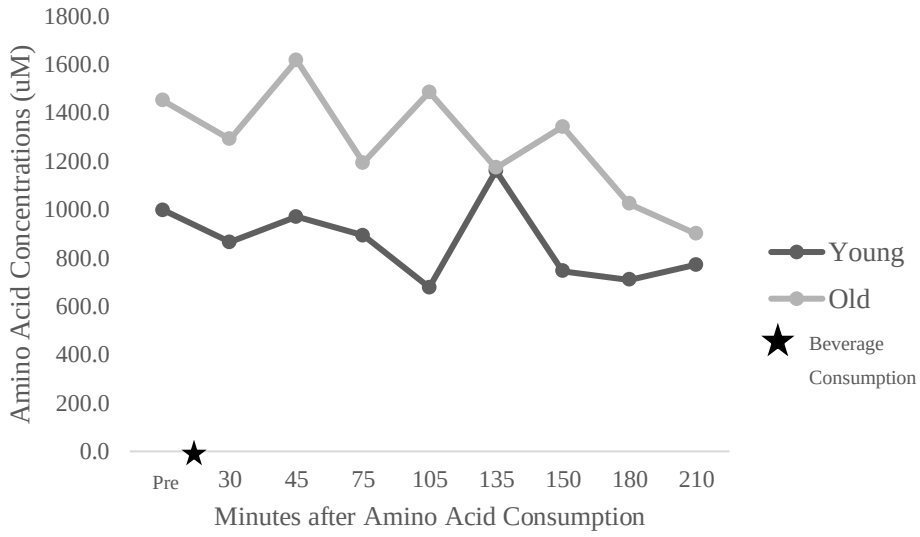


Figure 9: Total Amino Acid Concentrations in the Peritendinous Space

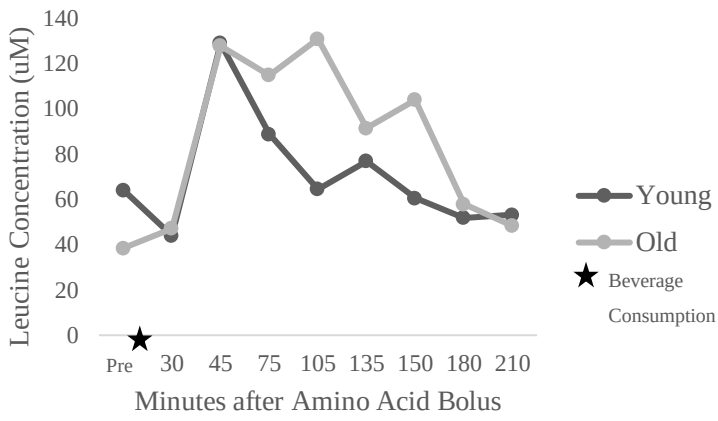


Figure 10: Concentration of Leucine in the Peritendinous Space Post-Supplementation

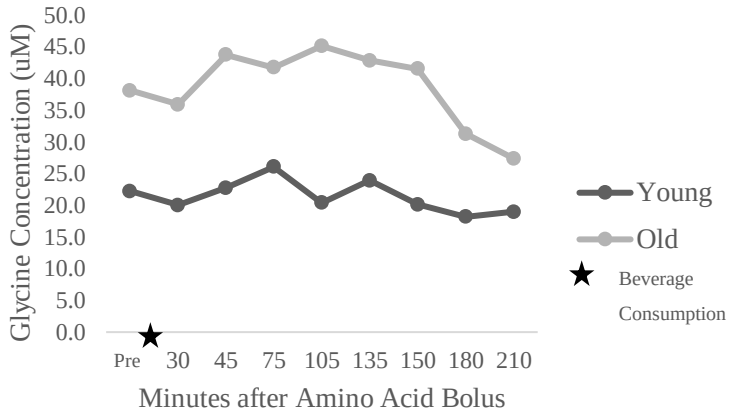


Figure 11: Concentration of Glycine in the Peritendinous Space Post-Supplementation

in Figure 7. Sarcosine was added to the saline perfusate solution as a recovery marker, and like proline, it requires a separate method of detection. With this being said, any sarcosine measured in the peritendinous space would have minimal implications on results and/or conclusions regarding amino acid delivery given that sarcosine was not a part of the supplemental beverage. Figures 8 and 9 show that the delivery of specific amino acids (leucine and glycine, respectively) was not impaired with age. Delivery of leucine was highlighted because it plays a significant role in muscle protein synthesis and was augmented in the supplemental beverage (Figure 8), whereas glycine is the main amino acid in collagen and was also augmented (Figure 9).

Procollagen Concentrations in the Peritendinous Space

Interestingly, basal concentrations of procollagen 1 alpha 1 (pg/mL) were higher in elderly versus young subjects. This difference appears to be statistically significant, but a larger sample size would be necessary to confirm this finding. Though it was unclear why the elderly had higher basal procollagen concentrations, this may be an indication of increased collagen turnover in aged tendon.

Building off of this theory, the relationship between the concentration of procollagen and particular amino acids was evaluated to determine if the increased prevalence of a given amino acid correlated with an increase in procollagen production. Similar to the assessment of amino acid delivery, procollagen concentrations in the peritendinous space were compared to that of leucine and glycine.

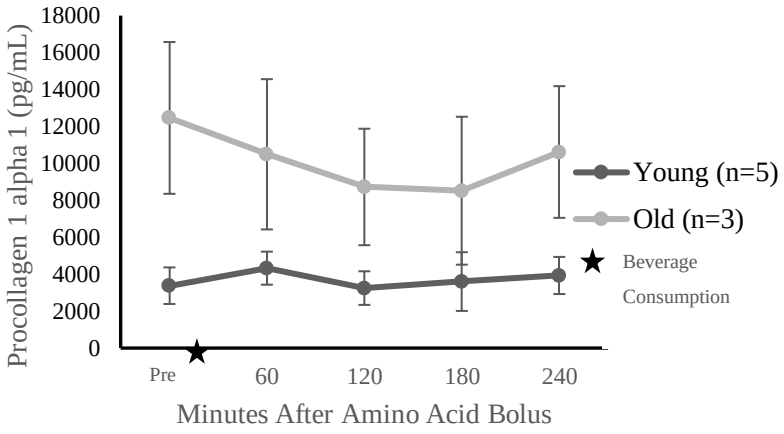


Figure 12: Procollagen Concentrations in the Peritendinous Space Post-Supplementation

The Enzyme-Linked Immunosorbent Assay (ELISA) was able to detect procollagen concentrations in the peritendinous space using the small sample volumes (10 uL) collected at each of the twenty time points, but the RP-HPLC required a greater sample volume in order to assess amino acid delivery to the peritendinous space. The ELISA was performed first with 10 uL per sample, but every two samples had to be pooled for the RP-HPLC analysis (the timepoint for each pooled sample was defined as the average of the two combined timepoints). For this reason, some procollagen concentrations were correlated to amino acid concentrations collected at slightly different timepoints.

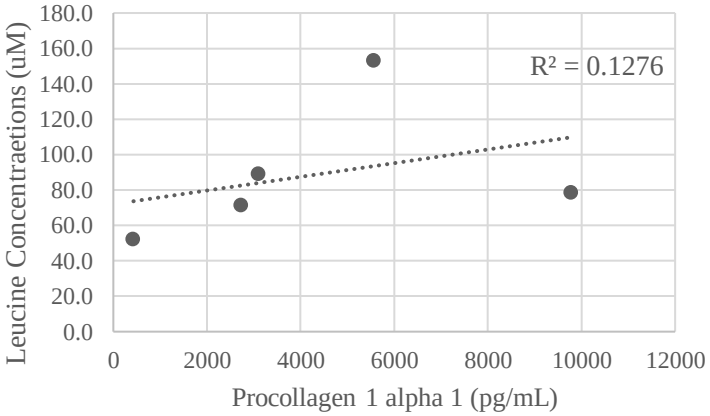


Figure 13: Correlation between Procollagen and Leucine Concentrations ~1 Hour Post-Supplementation in Young Subjects

Procollagen concentrations measured sixty minutes post-consumption did not correlate to leucine concentrations measured

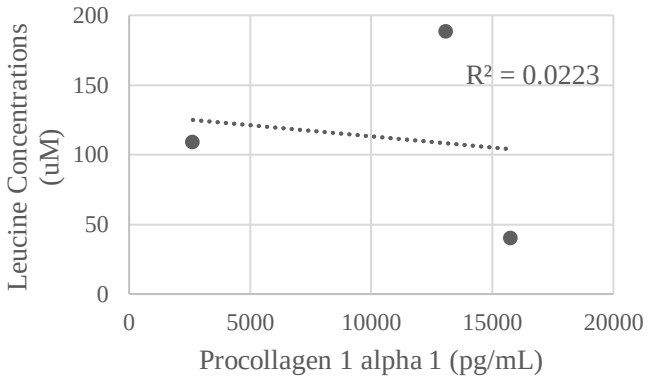


Figure 14: Correlation between Procollagen and Leucine Concentrations ~1 Hour Post-Supplementation in Older Subjects

seventy-five minutes post-consumption in young (R_2 = 0.1276, r = 0.357) nor older (R_2 = 0.0223) subjects. These findings are reflected in Figures 11 and 12, respectively.

Weak correlations between leucine and procollagen, however, were observed in young subjects at approximately two- and three-hours post-supplementation. The correlation coefficient between procollagen 120 minutes following amino acid supplementation and 135 minutes following leucine supplementation was 0.829 (R_2 = 0.6869), whereas the correlation between procollagen and leucine 180 minutes (3 hours) post-consumption was 0.772 (R_2 = 0.5958). Further analysis after adjusting for probe recovery and with a larger sample size is necessary to solidify these findings. Due to sample collection issues to be explained in the discussion, correlation coefficients could not be calculated for older subjects at these timepoints.

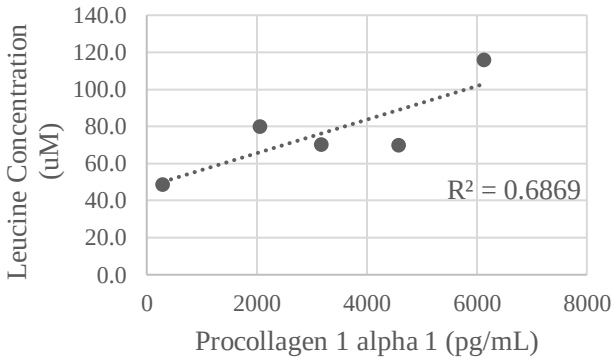


Figure 15: Correlation between Procollagen and Leucine Concentrations ~2 Hours Post-Supplementation in Young Subjects

Similar results were found when comparing procollagen and glycine concentrations in the peritendinous space one, two, and three hours post-supplementation. Glycine and procollagen concentrations did not correlate approximately sixty minutes after amino acid supplementation in young (Figure 15, R_2 = 0.1205, r = 0.347,) nor older subjects (Figure

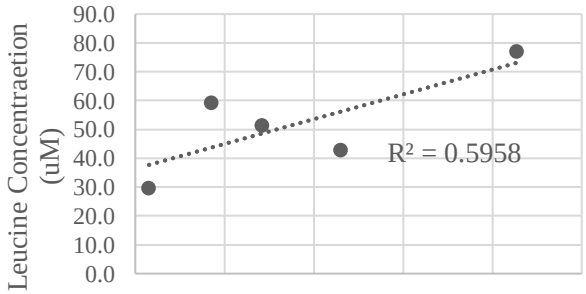


Figure 16: Correlation between Procollagen and Leucine Concentrations ~ 3 Hours Post-Supplementation in Young Subjects

16, R_2 = 0.0223, r = 0.149,). At approximately two-hours post-supplementation, there was a weak correlation between procollagen (120 minutes post-supplementation) and glycine (135 minutes post-supplementation) in the peritendinous space of young subjects (R_2 = 0.6563, r = 0.810). A similar, weak correlation was also observed three hours (180 minutes) post-supplementation in young subjects (R_2 = 0.603, r = 0.777). Unfortunately, due to RP-HPLC sample analysis issues, correlation coefficients could not be calculated for the elderly at these timepoints.

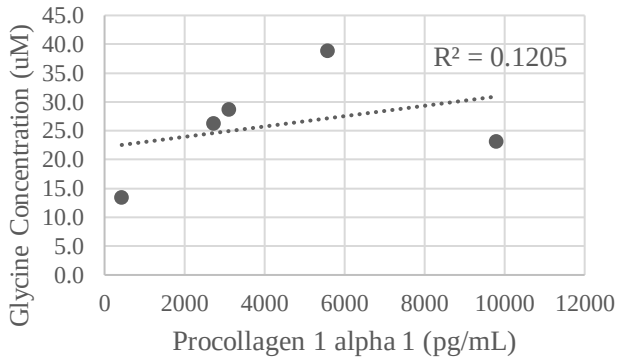


Figure 17: Correlation between Procollagen and Glycine Concentrations ~1 Hour Post-Supplementation in Young Subjects

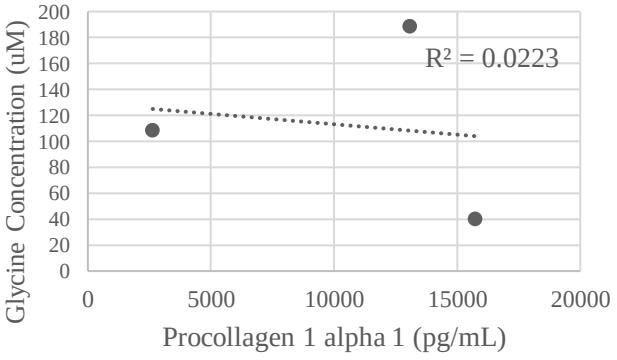


Figure 18: Correlation between Procollagen and Glycine Concentrations ~1 Hour Post-Supplementation in Older Subjects

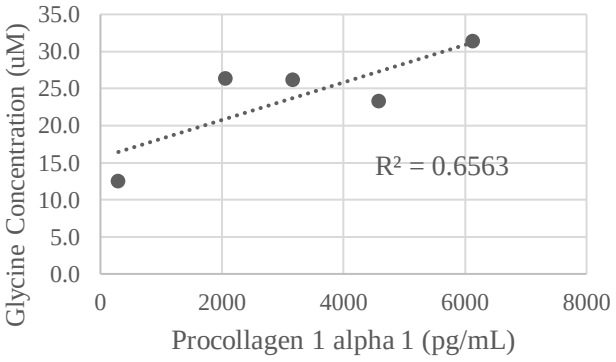


Figure 19: Correlation between Procollagen and Glycine Concentrations ~ 2 Hours Post-Supplementation in Young Subjects

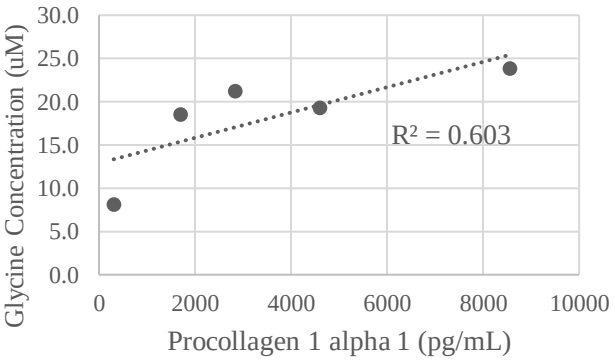


Figure 20: Correlation between Procollagen and Glycine Concentrations~ 3 Hours Post-Supplementation in Young Subjects

DISCUSSION

This study of amino acid delivery and procollagen synthesis is, based on our review of the current literature, the first to investigate the effect of supplemental amino acids on healthy, elderly individuals independent of an exercise intervention. The primary outcome – amino acid delivery to the peritendinous space – appeared to be increased in elderly compared to young subjects. This was contrary to our hypothesis that amino acid delivery would be unaffected by age. Given the time restrictions, though, only five young and five older subjects were analyzed; with a larger sample size and adjustments for probe recovery, this variability between young and old subjects will most likely decrease. Interestingly, basal procollagen concentrations were higher in the peritendinous space in old versus young subjects. Though the reason for this discrepancy is unclear, it is possible that the procollagen concentrations in the peritendinous space are elevated in the elderly due to increased collagen turnover.

Because factors that promote skeletal muscle anabolism are studied far more extensively than that for tendon, many tendon studies are predicated on mechanisms that, based on biologically-plausibility, many translate to tendon as well. Our study is no exception. In the long term, our research group hopes to investigate if amino acids can be taken up by tendons and utilized for remodeling. The hope is that this remodeling will lead to anabolic adaptations that improve the structural and functional properties of tendon. Assessing amino acid utilization was accomplished, in part, by measuring procollagen concentrations in the peritendinous space, but we felt that conducting this preliminary study to confirm that amino acid delivery is not impaired in the elderly was critical. Without a foundation to prove that supplemental amino acid delivery is comparable in young versus old subjects, any cause-and-effect relationships drawn on orally-consumed amino acids may be called into question due to speculated differences in amino acid bioavailability with age.

Research on the effect of amino acids on tendon is relatively sparse, but amino acids have been consistently shown to promote an anabolic response in muscle; given that muscle tissue is contiguous with that of tendon, the impact of amino acids on tendon should also be evaluated. The use of amino acids as a stimulus for tendon adaptations was further justified by the fact that tendons are composed of amino acid chains. By introducing more substances necessary for collagen synthesis, it is plausible to hypothesize that collagen synthesis may increase.

Peritendinous Microdialysis

Though the process is fairly straightforward, microdialysis relies heavily on the perfusate's ability to equilibrate with fluid in the extracellular space. This exchange is directly impacted by the perfusate's flow rate, the ability of the analytes to diffuse through the capillary wall, and properties of the dialysis membrane including its length, composition, and pore size[50–52]. Our saline-sarcosine solution was perfused through the catheter at a rate 2 μL/min, a rate that was specifically chosen to optimize the quality and time of our dialysate collection. A faster flow rate may not have allowed adequate time for solutes to exchange between the peritendinous space and the catheter membrane, while a slower flow rate would have limited the volumes of dialysate samples that could be collected in a reasonable amount of time. Because a flow rate of 2 μL/min has been successfully used in similar studies conducted by Dr. Carroll, we deemed it as an appropriate rate for the present investigation as well.

While using a suitable flow rate is key for optimal dialysate collections, sarcosine plays an equally important role in the overall success of microdialysis experimentation. Measures can be taken to promote optimal equilibration between the perfusate and the peritendinous extracellular fluid, but achieving absolute equilibrium is extremely difficult given the variety of aforementioned factors that impact exchange. In fact, Rosdahl et al. determined that either a long dialysis membrane (30 mm, 20,000 mol weight cut off) or a very low perfusion flow rate (0.16 μL/min) were necessary to achieve complete equilibrium in skeletal muscle or adipose tissue[51]. These conditions would be challenging to replicate given the location and duration of our microdialysis procedure. Furthermore, even if perfect exchange and thus absolute equilibrium were theoretically achieved, this could not be confirmed without having a marker of a known concentration.

In our study, sarcosine was used a recovery marker to correct for imperfect equilibrium. In other words, because flow rate, the diffusibility of the amino acids in the supplemental beverage, and the properties of the microdialysis catheter all impact how the saline perfusate solution equilibrates in the peritendinous space, it is possible that the collected dialysate contained only a fraction of the amino acid concentrations that were in fact delivered to the area around the tendon. This ratio of the amino acid concentrations in the dialysate to those in the extracellular, peritendinous fluid is referred to as 'recovery' and must be adjusted to obtain meaningful, accurate results[53]. Sarcosine is similar in size and structure to the supplemental amino acids provided in our beverage, which is key because these similarities suggest that sarcosine diffused into the

microdialysis catheter comparably to the supplemental amino acids. Moreover, because sarcosine was not provided in the supplemental beverage and is naturally present in negligible quantities in the extracellular space, we could assume with confidence that our sarcosine recoveries were not confounded by supplemental and/or endogenous amino acids. These similarities suggest that there is a linear relationship between the recovery of sarcosine and the recovery of the other supplemental amino acids in the dialysate, which allows us to approximate the concentration of the analyte in the peritendinous space[53]. If, for example, 40% of our known concentration of sarcosine was recovered in the dialysate, all other recoveries should be divided 40% to determine the actual concentrations of each amino acid in the peritendinous space. This calculation is known as the probe recovery adjustment.

Limitations

Several limitations of our study need to be considered when drawing conclusions from its findings. In terms of the participants, our subject criteria excluded anyone with a body mass index over 35 kg/m^2 or a history of diabetes mellitus, among other characteristics. Both obesity and diabetes are on the rise among elderly individuals[54,55] and are known as risk factors for tendinopathies[56], so implementing this criteria excluded a large subset of individuals who may theoretically benefit from amino acid supplementation. Additional investigations including these "unhealthy" subjects would be necessary to make more general claims about the effect of amino acids on elderly tendon.

Furthermore, data collection was impacted by the constraints of microdialysis perfusion. Subjects must be continuously tethered to the catheter and perfusion pump during the duration of the testing, and bathroom breaks are not possible without completely disconnecting the subject from the equipment. Because many subjects, especially the elderly, had to use the bathroom approximately one hour after consuming the supplemental amino acid beverage, several subjects were missing data points in the middle of their testing. The subject was transported to the bathroom (to prevent he/she from putting weight on his/her foot) and the clock remained running as if he/she had not been disconnected upon returning from the bathroom, but one to three data points were still lost each time a subject needed to use the restroom. It is also important to consider that our conclusions cannot be generalized to all amino acids because the study protocol did not vary the types nor concentrations at any point. Our beverage's composition was based on previous

literature[46], but these researchers were assessing skeletal muscle satellite cell proliferation, not tendon adaptations. Without further experimentation, it is unclear if tendons would respond differently to a specific set or threshold of amino acids.

Dialysate samples were collected every fifteen minutes with the intention of creating a precise, time-dependent curve of amino acid concentrations, but this was not possible given limitations during the RP-HPLC. In order to meet minimum sample volume requirements for each run, the following timepoints were combined: 3-4, 5-6, 7-8, 9-10, 11-12, 13-14, 15-16, 17-18, 19-20. Doubling the volume in each RP-HPLC run yielded the most accurate and replicable results with the samples that were collected, but in future studies, it would be better to collect larger samples at each collection point. The timepoint of each new, pooled sample was defined as the average of the times of the two samples that were combined, which posed a challenge when correlating the peritendinous concentrations of procollagen to that of either glycine or leucine. Procollagen concentrations, determined using the ELISA, were obtained for all fifteen-minute intervals as outlined in the original methodology. Amino acid concentrations at all twenty time points, however, could not be obtained due to RP-HPLC limitations/the need to pool samples. For this reason, some correlations between concentrations of procollagen and either leucine or glycine were drawn at similar but not precisely the same timepoint. This may have either strengthened or weakened the correlations calculated between the two.

Future Implications and Summary

Despite the drawbacks and constraints of the study, our findings are poised to serve as a strong foundation for future investigations. Given that amino acid delivery is not impaired with age, the next logical step would be to determine if aged tendon can efficiently take up and utilize these amino acids for remodeling. This pilot study demonstrates that amino acids delivery to the peritendinous space is not impaired with age, but it is still unclear if aged tendons are able to incorporate these amino acids into their structure. Additionally, if the elevated basal procollagen levels in the peritendinous space of elderly individuals is indicative of increased collagen turnover, it is reasonable to question why tendon collagen anabolism was targeted in this intervention. Tendon collagen synthesis may, in fact, be comparable to that of young subjects but overruled by a higher rate of degradation. Should experimentation prove this to be true, future interventions should instead aim to minimize degradation instead of promote synthesis in order to improve the

structure and function of aged tendon. It may also be worthwhile to study the impact of amino acid supplementation in the elderly with and without a concurrent exercise intervention. Studies show that the aged tendons to do not respond to exercise alone[6,39], but it would be interesting to determine if exercise in conjunction with amino acids improves tendon structure and/or function.

In summary, evidence suggests that amino acid delivery to the peritendinous space is increased in the elderly, but this variability will likely decrease with a larger sample size and adjustment for probe recovery. Additionally, procollagen concentrations in the peritendinous space do not correlate with glycine or leucine concentrations one hour post-supplementation in young or older subjects. There is, though, a weak correlation between procollagen and these amino acids in young subjects at approximately two and three hours post-supplementation. Our investigation was limited by procedural and circumstantial factors, but we nonetheless believe that this pilot study provides sufficient groundwork for future studies to further evaluate the impact of supplemental amino acids on the structure and function of aged tendon.

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