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Effects of Chronic Omega3- Supplementation on Tendon Structural and Mechanical Properties

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Abstract

Background: Omega-3 fatty acids have previously been shown to modulate collagen expression. Given that collagen protein is ubiquitous in connective tissue such as tendons, it is possible that omega-3 can also modify the composition of tendons, resulting in changes to its structural and mechanical properties.

Objective: Thus, the purpose of this study was to investigate the effects of chronic omega-3 supplementation on the structural and mechanical properties of the patellar tendon.

Design: Nine males (age: 21.3 ± 1.3) volunteered to take part in the study, where the structural and mechanical properties of the patellar tendon were assessed before and after a 4-week omega-3 supplementation period.

Results: There were no significant differences (p = 0.119, t = -1.744) in tendon length (pre 44.9 ± 3.6 mm vs. post 46.0 ± 3.3 mm, +2.5 % difference) or tendon cross-sectional area (p = 0.149, t = 1.621; pre 105.1 ± 57.1 mm2 vs. post 101.5 ± 55.2 mm2, -3.6 % difference) from pre to post-supplementation. Differences in tendon elongation (p = 0.488, t = -0.727; pre 5.0 ± 1.4 mm vs. post 5.5 ± 2.0 mm, +9 % difference), strain (p = 0.384, t = -0.920; pre 12.3 ± 3.9 % vs. post 13.8 ± 5.3 %, +11 % difference), stress (p = 0.103, t = -1.873; pre 55.4 ± 21.4 MPa vs. post 57.5 ± 22.8 MPa, +3.7 % difference), stiffness (p = 0.668, t = 0.445; pre 1674 ± 1208 N.mm-1 vs. post 1508 ± 397 N.mm-1, -11 % difference) and Young's modulus (p = 0.603, t = 0.544 pre 0.88 ± 0.76 GPa vs. post 0.77 ± 0.33 GPa, -14 % difference) were observed following the supplementation period, although these did not reach a level of significance).

Conclusions: Four weeks of omega-3 supplementation shows trends for reductions in the structural and mechanical properties of the patellar tendon. However, the supplementation period may not have been sufficiently long enough to show significant modifications of the patellar tendon structural and mechanical properties.

Introduction

Omega-3 fatty acids are a type of polyunsaturated fatty acid. The importance of omega-3 for physical well-being has been recognised for several decades [1]. Amongst other health benefits, omega-3 fatty acids have anti-inflammatory, antithrombotic, antiarrythmic and hypolipidaemic effects [2]. Thus, these fatty acids are considered to be beneficial with respect to a number of conditions ranging from coronary heart disease (3) to rheumatoid arthritis [4]. The omega-3 fatty acids include α-linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid, and docosahexaenoic acid (DHA) [5].

One of these omega-3 fatty acids, EPA, is abundant in fish oils and has been found to be associated with a number of physical health benefits, such as stimulating weight loss in obese subjects via increased metabolism and insulin sensitivity [6,7], as well as reducing the levels of pro-inflammatory cytokines associated with inflammation and muscle damage (delayed onset of muscle soreness) following a single bout of exercise [8-10].

In addition, EPA has also been shown to improve joint status and discomfort [11, 12]. Gruenwald et al. [11] reported reductions in joint stiffness, pain, and total number of swollen joints following 3 months of omega-3 supplementation in rheumatoid arthritis patients. Complimentary evidence is also provided by Caturla et al. [12] who found 9 weeks of omega-3 therapy to significantly reduce joint pain and stiffness as well as to increase physical function in subjects with joint discomfort. Thus, omega-3 fatty acids may be quite effective in the treatment of joint diseases such as osteoarthritis and rheumatoid arthritis in the sense of partially counteracting the disease-associated impairments in physical function. However, the specific mode of action for such an effect remains elusive. It is unclear as to whether the effects of omega-3 fatty acids on the joints are merely an increase in lubrication or whether the supplement has any direct impact on the intrinsic properties of the collagenous or elastic material of the tendon associated with the affected joint. Reports in the literature suggest that EPA has the ability to modulate the molecular pathways involved with collagen formation [13], switching on those genes involved with collagen production. Previous research examining the effects of EPA on skin has also reported collagen and elastic fibres (tropoelastin and fibrillin-1) to be up-regulated via increased transformin growth factor-β (TGF-β) expression [14].

The suggestion that EPA may induce changes in collagen is of particular interest from a muscle function perspective because collagen protein is ubiquitous in connective tissues such as tendons. At the cellular level, tendons are primarily composed of type I collagen fibres which possess certain mechanical properties that have important implications for muscle function and injury risk [15]. Stiffness is a functional property of the tendon which is principally associated with the unit's ability to transfer forces; a stiffer tendon being able to transfer the muscle forces to the bone more rapidly than a less stiff (i.e., more compliant) tendon [16]. The level of stiffness/compliance is also known to affect both the length of the contractile component and the shortening velocity of muscle, which in turn would directly influence the force generated [17,18]. Strain is another important characteristic of the tendon which can be defined as a measure of the deformation of the tendon upon force application relative to the resting length of the same unit, and is often used as an indicator of injury risk. Indeed a tendon that deforms continuously under load may reach its elastic limit and rupture via excess strain [19,20].

The mechanical properties of the tendon are affected by its composition, in that a greater density of collagen for example will result in a higher stiffness value. As EPA supplementation has been shown to increase collagen and elastic fibre expression [14], it is possible that it could also affect the mechanical properties of the tendon. However, to date, no data exists relating to the effects of chronic EPA supplementation on tendon properties. Owing to the above outlined importance of tendon properties in aspects of muscle function, any alteration in its composition could have implications for performance, daily function and injury risk. Therefore, in light of fish oil supplements being used extensively for potential health benefits, further knowledge of their action on tendon tissue is of significant importance.

Thus, it is the purpose of this study to investigate the effects of chronic EPA ingestion on the patellar tendon structural and mechanical properties. Owing to its known affect on collagen and elastic fibre expression, it is hypothesised that omega-3 supplementation will have an effect on the tendon properties.

Subjects and Methods

Subjects and Experimental Design

Nine recreationally active males (people accustomed to exercising up to 3 times weekly) aged (mean ± standard deviation (SD)) 21.3 ± 1.3, with a height of 178 ± 2.5 cm, and a mass of 74 ± 3.5 kg, volunteered to take part in the study. None of the included participants had a history of injury to the examined areas. All participants were instructed to carry out their normal activities as previous to the administration of the fish oils. All subjects gave their written informed consent to participate and the study had the approval of the local University Ethics Committee. The study was also in agreement with the World Medical Association's declaration of Helsinki describing ethical principles for medical research involving human subjects. The study was a test, re-test design in which the structural and me-

chanical properties of the patellar tendon were assessed pre and post a 4-week omega-3 fish oils supplementation period. All participants had a familiarisation session in the laboratory prior to any testing and all testing was undertaken following a standardised warm-up.

Measurement of Patellar Tendon Forces

All measurements of torque were carried out on an isokinetic dynamometer (KinCom dynamometer, type 125 AP, Chattanooga, USA). The knee was fixed at 90º flexion (full extension = 0º) and hip at 85º (supine = 0º). The centre of rotation of the dynamometer lever arm was aligned with the knee joint centre, and straps were fixed across the chest, hip, and thigh of the test limb to prevent any extraneous movement. A lever attachment cuff was placed on the lower leg at ~3 cm above the medial malleolus. Prior to testing, three maximal isometric knee extension efforts were carried out to ensure tendon preconditioning. Participants were then required to perform ramped isometric knee extensions to maximum over a 3 - 4 s time period. Three trials of the test were performed with 180 s rest between contractions. Tendon force was calculated as Ftend = (P + Pantag)/Tarm, where Ftend = force in the patellar tendon, P = observed knee extensor torque output, Pantag = antagonistic (hamstring) co-contraction torque, and Tarm = patellar tendon moment arm 44.7 ± 1.6 mm computed from the average of previous reports [20, 21].

Estimation of Hamstring Co-contraction

Hamstring torque was estimated by using electromyography (EMG). The EMG of the long head of the biceps femoris muscle (BF) was measured in order to ascertain the level of antagonistic muscle co-contraction [18, 22-25] during the isometric knee extension efforts. Assumptions were that BF is representative of its constituent muscle group [26], and BF EMG relationship with knee flexors torque is linear [27, 28]. A pair of self-adhesive Ag-AgCI electrodes ~15 mm in diameter (Medicotest, Rugmarken, Denmark, type N-10A), were placed in a bipolar configuration with a constant between –electrodes distance of ~20 mm, at the distal one-third of the length, in the mid-line of the belly of the BF. Prior to electrode attachment the skin was prepared by shaving, abrading, and cleaning with an alcohol-based solution in order to minimise the resistance. The reference electrode (Type Q-10A) was placed on the lateral tibial condyle of the test limb. The raw EMG signal was sampled at 2000 Hz, pre-amplified (x2000, Neurolog remote AC preamplifier, type NL 822, Digitimer, UK), amplified (x2) (Neurolog isolation amplifier NL 820, Digitimer, UK) and band pass filtered between 10 Hz and 500 Hz (Neurolog, type NL 134 and NL 144, Digitimer, UK). All EMG and torque signals were displayed in real time Testpoint software (CEC, MA, USA) via a PC. A series of three maximal isometric knee flexion contractions were carried out to obtain the EMG value at maximal flexion torque. The root mean square (RMS) EMG activity corresponding to the peak torque period was analysed over 50 ms epochs and averaged for a 1 s period during the plateau of peak torque. This has previously been suggested to be acceptable in terms of signal-to-noise ratio [29]. EMG activity of the BF during knee extension was divided by the maximal BF flexor EMG, and the maximal flexor torque was then multiplied by this value to determine co-contraction torque.

Measurement of Patellar Tendon Structural and Mechanical Properties

Force-Elongation Relationships

Tendon force-elongation relationships were determined using a method previously described [18, 23, 24]. Briefly, simultaneous recordings of the patellar excursions, generated torque and EMG were carried out during the ramped isometric knee extensions. Patellar tendon images were generated using B-mode ultrasound (AU5, Esaote, Genoa, Italy) with a 7.5 MHz linear array probe (100 mm wide) with a depth resolution of 67 mm. The probe was positioned in the saggital plane over the patellar tendon covering both the apex of the patellar (proximal end/origin) and the tibial tuberosity (distal end/insertion) to account for total tendon elongation. Tendon images were digitised directly to a PC at 25 frames s^{-1} (Adobe Premier pro Ver.2) and synchronised (using an electronic signal generator) with the force and EMG records to allow temporal alignment. Tendon elongation was determined at every 10% interval of the maximal voluntary contraction (MVC) force (10 – 100%) using image J (National Institute of Health, Bethesda, MD, USA).

Tendon Stiffness

The tendon force-elongation relationships were fitted with second order polynomial functions forced through zero. Tendon stiffness (K) measures (in $N.mm^{-1}$) were then calculated from the slope of the tangents at 10% force intervals.

Tendon Length and Tendon Cross-sectional Area

Patellar tendon cross-sectional area (TCSA) and resting tendon length (TL) were assessed with the knee joint angle at 90º. TCSA was measured from the transverse-plane ultrasound images taken at the average of 0, 25, 50, 75, and 100% of TL. TL was determined from saggital-plane ultrasound images and measured from the inferior pole of the patellar to the superior aspect of the tibial tuberosity.

Tendon Tensile Modulus or Young's Modulus

Tensile modulus, hereafter referred to as Young's modulus (GPa), was computed as the product of K and the ratio between

TL to TCSA.

Tendon Strain and Tendon Stress

Tendon strain (%) was calculated as the ratio of tendon elongation to the TL. Tendon stress (MPa) was calculated by dividing force in the tendon by TCSA.

Supplementation

All subjects were required to take supplemental omega-3 fish oils for a 4-week period following their baseline test. The specific dosage taken by the subjects was 4.2 g of omega-3 fish oils per day, of which 2.16 g was EPA and 1.44 g was DHA.

Statistical Analyses

Firstly, data were checked for normality using the Shapiro-Wilks test. Paired Student t-tests were then used to examine the effects of omega-3 supplementation on tendon properties. Intraclass correlation coefficients (ICCs) (one-way random effects model) were also computed to determine reliability of the measures. Alpha level was set to $p < 0.05$. All data are presented as mean ± SD.

Results

The within-session ICCs at baseline were 0.976 for tendon force, 0.742 for tendon elongation, 0.999 for TL and 0.986 for TCSA, respectively. The ICCs post-intervention were 0.980 for tendon force, 0.837 for tendon elongation, 0.992 for TL and 0.869 for TCSA, respectively.

The statistical analyses revealed no significant differences in either TL (pre 44.9 ± 3.6 mm vs. post 46.0 ± 3.3 mm, +2.5 % difference, p = 0.119, t = -1.744) or TCSA (pre 105.1 ± 57.1 mm2 vs. post 101.5 ± 55.2 mm2, -3.6 % difference, p = 0.149, t = 1.621) from pre to post supplementation.

The tendon force-elongation relationships were fitted with second order polynomials (Figure 1) which illustrate the differences in patellar tendon excursion before and after 4 weeks of omega-3 ingestion. No significant changes were observed from pre to post-supplementation for maximal tendon elongation (pre 5.0 ± 1.4 mm vs. post 5.5 ± 2.0 mm, +9 % difference, p = 0.488, t = -0.727) or maximal tendon strain (pre 12.3 ± 3.9 % vs. post 13.8 ± 5.3 %, +11 % difference, p = 0.384, t = -0.920). However with Figure 1 in mind, a trend can be seen for greater elongation and strain at all levels of force following omega-3 supplementation as the force-elongation curve is shifted to the right, indicating that at similar levels of force experienced by the tendon, elongation is consistently greater. Similarly, there were also no significant changes in maximal tendon stress from pre to post supplementation (pre 55.4 ± 21.4 MPa vs. post 57.5 ± 22.8 MPa, +3.7 % difference, p = 0.103, t = -1.873), although there were trends suggesting minor increases (Figure 2). The force-elongation (Figure 1) and stress-strain (Figure 2) graphs illustrate the loading profiles of all subjects before and after 4 weeks of omega-3 supplementation.

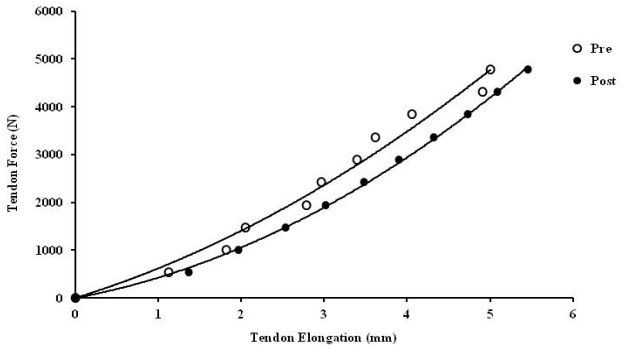

Figure 1. Patellar tendon force-elongation relationships. Open markers represent baseline measurements and closed markers represent measurements following 4 weeks of omega-3 ingestion. At all force levels, tendon elongation is consistently greater post-supplementation compared to the values at baseline ($p > 0.05$).

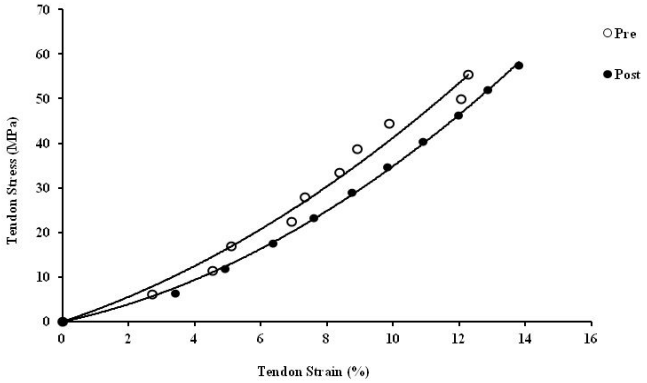

Figure 2. Patellar tendon stress-strain relationships. Open markers represent baseline measurements and closed markers represent measurements following 4 weeks of omega-3 supplementation. Tendon strain and tendon stress are consistently greater post supplementation at all levels of force compared to the values at baseline ($p > 0.05$).

Tendon stiffness values calculated from the force-elongation curves (see methods for details) were consistently different across the absolute force levels identified. There were no significant changes in tendon stiffness from pre to post supplementation at any relative force level (at 100% MVC, pre 1674 ± 1208 $N.mm^{-1}$ vs.post 1508 ± 397, p = 0.622, t = 0.512). However, there were trends suggesting consistent decreases following 4-weeks of supplementation at all levels of force, with a

mean of 1332 ± 913 N.mm^{-1} and 1152 ± 264 N.mm^{-1} (-16 % difference) at pre and post-supplementation, respectively, in terms of mean stiffness across all levels of force.

Interestingly, a pattern was also observed for the changes in tendon stiffness from pre to post supplementation, whereby as the force level increased, the magnitude of change in tendon stiffness declined (at 10% MVC, pre 970 ± 766 N.mm^{-1} vs. post 700 ± 218 N.mm^{-1}, -39 % difference; at 100 % MVC, pre 1674 ± 1208 N.mm^{-1} vs. post 1508 ± 397 N.mm^{-1}, -11 % difference). Thus, a "cut-off point" (60 % MVC) was identified which related to a specific pattern of the changes in tendon stiffness with omega-3 supplementation (Figure 3). The greatest decrements in tendon stiffness were exhibited below this force level, whereas above this point tendon stiffness changes were minimal.

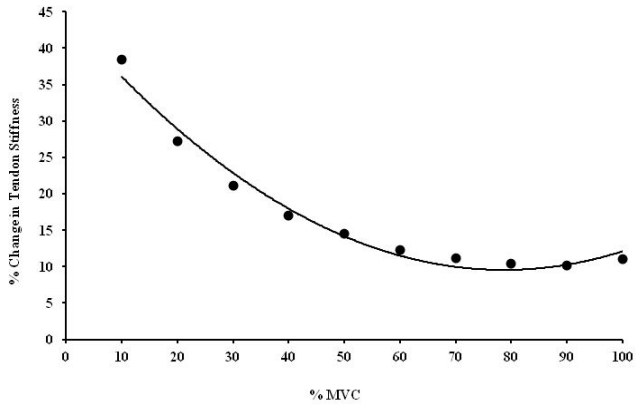

Figure 3. The magnitude and character of tendon adaptation with omega-3 therapy is force-region specific. As force level is increased, the magnitude of decrements in tendon stiffness decline. There is a trend emerging whereby the tendon exhibits the greatest change in stiffness with omega-3 supplementation at low forces (<60 % MVC). Above this level, however, the changes are stabilised and minimal.

Moreover, in order to account for any differences in tendon dimensions from pre to post supplementation, Young's modulus was also calculated which normalises tendon stiffness values for TL and TCSA. Similarly to tendon stiffness, however, there were no significant changes in Young's modulus between pre and post supplementation at any relative force level (Figure 4), although there were trends of consistent decreases at all levels of force post supplementation (at 100 % MVC, pre 0.88 ± 0.76 GPa vs. post 0.77 ± 0.33 GPa, -14 % difference, p = 0.603, t = 0.544). Table 1 displays the patellar tendon mechanical properties at all force levels before and after 4 weeks of omega-3 supplementation.

In addition, to account for differences in maximal force, a standardised force level of 2950 N (corresponding to the maximal force of the weakest participant) was used to compare the pre and post-supplementation data sets. Tendon elongation (pre 3.6 ± 1.4 mm vs. post 3.8 ± 1.1 mm, +5.3 % difference, p = 0.591, t = -0.559), strain (pre 8.8 ± 3.6 % vs. post 9.6 ± 2.7 %, +8.3 % difference, p = 0.651, t = -0.470), and stress (pre 32.9 ± 11.3 MPa vs. post 33.9 ± 11.4 MPa, +3 % difference, p = 0.237, t = -1.292) were all found to increase from pre to post supplementation. Tendon stiffness (pre 1366 ± 817 N.mm^{-1} vs. post 1255 ± 817 N.mm^{-1}, -9 % difference, p = 0.668, t = 0.445) and Young's modulus (pre 0.76 ± 0.56 GPa vs. post 0.63 ± 0.25 GPa, -21 % difference, p = 0.422, t = 0.853) at the standardised force level of 2950 N were found to decrease between pre and post supplementation.

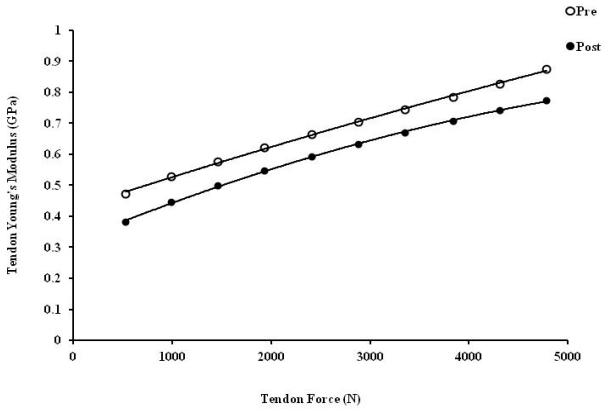

Figure 4. Young's modulus as a function of incrementing level of voluntary tendon forces and the effects of omega-3 supplementation. Open markers represent baseline measurements and closed markers represent measurements following 4 weeks of omega-3 ingestion. At all levels of force, Young's modulus is consistently lower post supplementation compared to the values at baseline (p > 0.05).

Discussion

The purpose of this study was to investigate the effects of chronic omega-3 supplementation on the structural and mechanical properties of the patellar tendon. The main findings suggest that there were no significant changes in the structural or mechanical properties from pre to post-supplementation (p > 0.05), and thus, the hypothesis is rejected. However, a trend can be seen for greater values of tendon elongation, strain and stress, and reduced values of stiffness and Young's modulus at equivalent forces following the supplementation period.

The health benefits of omega-3 fish oils and their constituent fatty acids, EPA and DHA, have been studied extensively. In most cases the effects are seen compatible with improvements in disease biomarker profiles or in health-related outcomes [30, 31]. Evidence suggests that optimising intake of omega-3 fatty acids may confer many benefits including reduced risk of cardiovascular disease [32], inflammatory disease [4] and

Table 1. Effects of 4 weeks of omega-3 supplementation on the in vivo patellar tendon mechanical properties

Force (%)	Force (N)	Pre-test					Post-test				
		Elongation (mm)	Strain (%)	Stress (MPa)	Stiffness ($N.mm^{-1}$)	Young's Modulus (GPa)	Elongation (mm)	Strain (%)	Stress (MPa)	Stiffness ($N.mm^{-1}$)	Young's Modulus (GPa)
~10	530	1.1 ± 0.8	2.7 ± 1.9	6.2 ± 2.8	970 ± 766	0.47 ± 0.47	1.4 ± 1.2	3.4 ± 2.9	6.4 ± 2.9	700 ± 218	0.38 ± 0.16
~20	991	1.8 ± 1.0	4.6 ± 2.6	11.5 ± 4.5	1064 ± 773	0.53 ± 0.48	2.0 ± 1.3	4.9 ± 3.2	12.0 ± 4.8	837 ± 199	0.44 ± 0.17
~30	1464	2.1 ± 1.1	5.1 ± 2.6	17.0 ± 6.5	1150 ± 790	0.58 ± 0.50	2.6 ± 1.4	6.4 ± 3.4	17.7 ± 6.9	950 ± 209	0.50 ± 0.19
~40	1938	2.8 ± 1.2	6.9 ± 3.2	22.5 ± 8.6	1228 ± 820	0.62 ± 0.52	3.0 ± 1.5	7.6 ± 3.7	23.4 ± 9.2	1050 ± 233	0.55 ± 0.21
~50	2410	3.0 ± 1.5	7.3 ± 3.7	27.9 ± 10.8	1305 ± 855	0.66 ± 0.54	3.5 ± 1.6	8.8 ± 3.9	29.0 ± 11.4	1139 ± 260	0.59 ± 0.23
~60	2885	3.4 ± 1.6	8.4 ± 4.0	33.4 ± 12.9	1375 ± 904	0.70 ± 0.58	3.9 ± 1.6	9.9 ± 4.2	34.7 ± 13.7	1224 ± 291	0.63 ± 0.26
~70	3349	3.6 ± 1.5	8.9 ± 3.9	38.8 ± 15.0	1445 ± 957	0.74 ± 0.61	4.3 ± 1.7	10.9 ± 4.5	40.3 ± 15.9	1299 ± 319	0.67 ± 0.28
~80	3840	4.1 ± 1.4	9.9 ± 3.6	44.5 ± 17.3	1518 ± 1020	0.79 ± 0.65	4.7 ± 1.8	12.0 ± 4.8	46.3 ± 18.4	1374 ± 1347	0.71 ± 0.29
~90	4309	4.9 ± 1.6	12 ± 4.3	49.9 ± 19.4	1591 ± 1098	0.83 ± 0.70	5.1 ± 1.9	12.9 ± 5.0	51.9 ± 20.6	1444 ± 373	0.74 ± 0.31
~100	4778	5.0 ± 1.4	12.3 ± 3.9	55.4 ± 21.4	1674 ± 1208	0.88 ± 0.76	5.5 ± 2.0	13.8 ± 5.3	57.5 ± 22.8	1508 ± 397	0.77 ± 0.33
Mean across all force levels		3.2 ± 1.2	7.8 ± 3.1	30.7 ± 16.6	1332 ± 913	0.68 ± 0.13	3.6 ± 1.6	9.1 ± 4.0	31.9 ± 17.2	1152 ± 264	0.60 ± 0.13

Force (%) represents the closest round number to the relative force level at which individual data was calculated. Force (N) represents the absolute group mean data corresponding to these relative force levels. Thus, tendon elongation, strain, stress, stiffness and Young's modulus were calculated at the same absolute force values to allow a direct comparison of tendon mechanical properties pre and post omega-3 supplementation. Data are mean ± SD.

possibly reduce the likelihood of behavioural problems and improve mental health [2]. To the authors' knowledge, however, this is the first study to examine the effects of omega-3 fatty acids on tendon properties.

Within the muscle-tendon unit, tendons are viscoelastic tissues of particular interest as they are functionally crucial to skeletal muscle contractile characteristics and as such influence all manners of tasks of daily motor performance [33]. In order for a muscle to develop force, it must first take up the slack within the tendon. Thus, all factors being equal (i.e., tendon dimensions (length and cross-sectional area), muscle architecture, length-tension, and force-velocity properties), stiffer tendons would enable a more rapid force transmission to the bones and are thus able to facilitate high rates of force production due to less slack within the system. In support of this, tendon stiffness has also previously been associated with rate of force development [34]. This may be particularly important in elderly populations where there is a high incidence of falls [35]. In this sense, a stiffer tendon would allow a more rapid force production and thus may increase the speed at which the muscle-tendon unit corrects the 'catch and throw' actions involved in maintaining balance and preventing a fall [36, 37]. This has also been shown by Onambele et al. [37] who found tendon stiffness to be associated with increased balance capabilities in the elderly.

The current study found no significant changes in tendon properties following 4 weeks of omega-3 supplementation. However, there were some trends suggesting increased tendon elongation (at 100 % MVC, pre 5 mm vs. post 5.5 mm, +9 % difference), strain (at 100 % MVC, pre 12.3 % vs. post 13.8 %, +11 % difference) and stress (at 100 % MVC, pre 55.4 MPa vs. post 57.5 MPa, +3.7 % difference), and decreased tendon stiffness (at 100 % MVC, pre 1674 N.mm-1 vs. post 1508 N.mm-1, -11 % difference) and Young's modulus (at 100 % MVC, pre 0.88 GPa vs. post 0.77 GPa, -14 % difference) following the supplementation period. It is thus speculative whether significant differences would have been observed had the supplementation period been longer than 4 weeks or had the dosage been greater than 4.2 g per day. However, it is difficult to make any definitive judgments due to the dearth of research in this area. Indeed, the decrements in Young's modulus indicate that the reduced tendon stiffness post supplementation is not due to alterations in the structural dimensions of the tendon but due to changes in its intrinsic properties. Thus, the findings of the

current study may also provide an additional explanation for previous work that has found omega-3 to reduce joint stiffness and pain in subjects with joint diseases [11, 12]; in that omega-3 therapy does not merely increase lubrication around a joint but may also modulate the collagenous or elastic material of the tendon associated with the affected joint. This notion may be of particular value to future researchers in establishing the specific mode of action for the therapeutic benefits of omega-3 fatty acids in the treatment of joint disorders, although it cannot fully be validated from the findings of this study due to the absence of statistical significance.

Owing to the uniqueness of the current work, it has generally been inappropriate to make direct comparisons with previous studies. Indeed Kim et al. [14] reported EPA to be associated with increased collagen and elastic fibre expression in aged human skin. However, these authors utilised the topical application of EPA for 2 weeks and not the use of oral supplements as that used in the current study. It was then reported by Kim et al. [14] that EPA has the ability to up-regulate collagen and elastic fibres (tropoelastin and fibrillin-1) via increased TGF-β expression. TGF-β is a multifunctional cytokine that plays an important role in the synthesis of extracellular connective tissue. It is also known to stimulate the proliferation of fibroblasts and to induce the synthesis and secretion of extracellular matrix proteins such as collagen, tropoelastin and fibrillin-1 [38-40]. Kim et al. [14] also found EPA to increase the expression of TGF-β1, -β2, and -β3, respectively. Collectively, these findings indicate that EPA may increase the expression of collagen and elastic fibres by increasing the levels of TGF-β1, -β2, and -β3. Based on these considerations, it is possible that EPA can also modulate the collagen and elastic content within tendinous tissues, and hence, change its mechanical properties although owing to the findings of the current study, a supplementation period greater than 4 weeks or a dosage greater than 4.2 g per day may be required in order for the extracellular matrix of the tendon to be significantly altered.

In addition, given that EPA has been shown to increase collagen expression [14], and that a greater density of collagen within tendons may result in a higher stiffness value, one may speculate that tendons become stiffer with omega-3 therapy, which is not in line with the findings of the current study. However, it must be noted that collagen expression has only been shown to increase with EPA [14] whereas a combination of EPA and DHA were used in the present study. "Thus, for future studies it may be useful to examine more specifically any effects of combined DHA and EPA on the elastic properties of tendons to determine the potential mechanisms for the increased tendon compliance with Omega-3 therapy indeed, Kim et al. [14] also found EPA to increase the expresion of elastic fibres in skin." Though, given that elastic fibres only account for 1-2% of the dry mass of the tendon [41, 42] it is unlikely that any increase in elastic fibre expression with omega-3 therapy will be responsible for increasing tendon compliance. On the other hand, it must be noted that other factors in the extracellular tendon matrix such as the tendinous ground substance which surrounds the collagen (consisting of proteoglycans, glycosaminoglycans and structural glycoproteins) can also influence the elasticity of the tendon component. More specifically, it has been reported that proteoglycans and glycosaminoglycans (macromolecules of the ground substance) have the capacity to improve the biomechanical properties (elasticity) of a tendon against shear and compressive forces [42]. They are also important for stabilisation of the whole collagenous system of connective tissue and for maintenance of ionic homeostasis and collagen fibrillogenesis [42]. Thus, there is a potential that in the current study the omega-3 fatty acids had a specific influence on the different components of the extracellular tendon matrix (i.e., collagen fibres, elastic fibres, ground substance), possibly reducing collagen turnover and increasing elastic fibre expression as well as modulating the tendinous ground substance, ultimately causing the tendon structure to become more compliant. However, this is beyond the scope of the current study to determine and thus requires further research.

The findings of this investigation indicate that the patellar tendon may become more compliant (i.e., greater elongation and strain, reduced stiffness) with chronic omega-3 supplementation. This may perhaps indicate a possible disadvantage with respect to function, muscle output and injury risk. All things being equal (i.e., tendon dimensions (length and cross-sectional area), muscle architecture, length-tension, and force-velocity properties), a more compliant tendon would be expected to allow a greater degree of sarcomere shortening as well as a faster shortening velocity of muscle, both of which may adversely affect force production [17, 18], and thus be detrimental with respect to muscle output and daily function. In addition, a compliant tendon would also lend itself to greater tendon deformation for equivalent forces, thus increasing the potential for large strain values and subsequent tendon-related injuries. However, despite the potentially deleterious effects of the direction of changes in tendon mechanical properties with omega-3 supplementation, previous research also suggests that compliant tendons may be beneficial in some aspects of function [16]. In fact, a review by Pearson & McMahon [16] highlighted that the effects of musculotendinous stiffness on performance are subject to the specific mode of task performed. The authors reported that where high rates of force production are required, a relatively stiff tendon may be optimal. Indeed, this notion is also supported by many reports in the literature that have found increased stiffness to be associated with increased sprint [44, 45], jump [46], and balance capabilities [37], all of which require high rates of force production. However, Pearson & McMahon [16] also suggested that where efficiency or economy is the main concern (i.e., long, continuous activities), greater compliance may enable more energy storage and release, and thus help reduce the work

done by the muscle. In general agreement with this is the work of Anderson & Pandy [43] who found increased series compliance to be associated with greater storage and release of elastic energy, which then translated into increased efficiency of jump performance, reducing the effective work of the muscles. Moreover, a tendon can also act as a shock absorber whereby possessing greater compliance can lead to the damping of high and potentially injury-inducing forces, for instance when landing in running or jumping. Thus, it appears that compliant tendons can also be beneficial in certain scenarios; nevertheless, it is as yet unclear whether the omega-3-induced changes in tendon properties would promote a positive physiological outcome on human functional performances, as this remains to be examined.

A possible limitation to this study may be the relatively specific and small sample of subjects used, who were in fact all males. It should be noted here that only males were selected for the study because female tendons are slightly more complex, in that they have also been shown to be influenced by circulating hormonal factors such as oestrogen and/or progesterone [47], which would additionally need to be accounted for in the study design. However, it may be interesting to see whether there is a similar effect on female tendons with omega-3 supplementation. Also, given that females naturally have more compliant tendons compared to their male counterparts [48], it may be that the magnitude of alteration in tendon mechanical properties with omega-3 therapy is different between the sexes, although this is yet to be investigated.

In conclusion, the results of this study show no significant changes in the structural and mechanical properties of the patellar tendon following 4 weeks of omega-3 ingestion ($p > 0.05$), although small changes were present indicating that the tendon may become more compliant with omega-3 therapy. Future studies should (a) examine the effects of omega-3 fatty acids on tendon properties using either a supplementation period greater than 4 weeks or a dosage greater than 4.2 g per day to determine whether the supplement significantly influences tendon properties, and (b) investigate the effects of the omega-3 induced changes in tendon properties on functional performances. Larger scale studies with randomised controlled trials and more heterogeneous samples would also allow for a more generalised data set to be generated.

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Author's Contributions to Manuscript

The author's responsibilities were as follows: SJP and SRH designed the research, conducted the research, analyzed data, wrote the paper, and had primary responsibility for final content. None of the authors had a conflict of interest.

List of Abbreviations

EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, TGF-β: transformin growth factor-β, EMG: electromyography, BF: biceps femoris, MVC: maximal voluntary contraction, TL: tendon length, TCSA: tendon cross-sectional area, K: tendon stiffness

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