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Mild untreated hypercholesterolaemia affects mechanical properties of the Achilles tendon but not gastrocnemius muscle

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ABSTRACT

Tendon xanthoma and altered mechanical properties have been demonstrated in people with familial hypercholesterolaemia. However, it is unclear whether mild, untreated hypercholesterolaemia alters musculotendinous mechanical properties and muscle architecture. We conducted a case-control study of adults aged 50 years and over, without lower limb injury or history of statin medication. Based on fasting low-density lipoprotein (LDL) cholesterol levels, 6 participants had borderline high LDL (>3.33 mmol/L) and 6 had optimal LDL cholesterol (<2.56 mmol/L). Using shear wave elastography, shear wave velocity (SWV) of the Achilles tendon and gastrocnemius medialis muscle (a proxy for stiffness), along with muscle fascicle length and pennation angle were measured under four passive tensile loads (0, 0.5, 1.0, 1.5 kg) applied via a pulley system. Differences between groups were found for tendon SWV but not muscle SWV, fascicle length or pennation angle. Participants with hypercholesterolaemia showed greater SWV (mean difference, 95 % CI: 2.4 m/s, 0.9 to 4.0, $P = 0.024$) compared to the control group across all loads. These findings suggest that adults with mild hypercholesterolaemia have increased tendon stiffness under low passive loads, while muscle was not affected. Future research is needed to confirm findings in a larger cohort and explore the impact of hypercholesterolaemia on tendon fatigue injury and tendinopathy.

1. Introduction

Hypercholesterolaemia is highly prevalent, particularly in westernised societies, with more than one in seven adult men and women having raised circulating levels of total and low-density lipoprotein (LDL) cholesterol (Timmis et al., 2018). Extensive research shows that raised cholesterol levels, even mildly elevated LDL levels, are associated with atherosclerosis and calcified coronary plaque (Won et al., 2019). In contrast, much less is known about the effects of hypercholesterolaemia on the musculoskeletal system, despite it being a significant risk factor for tendon pathology (Tilley et al., 2015) and tendon rupture (Mathiak et al., 1999, Ozgurtas et al., 2003).

In people with hypercholesterolaemia secondary to a genetic disorder (e.g., familial hypercholesterolaemia), lipids accumulate within the tendon extracellular matrix and form deposits called xanthomas (Soslowky and Fryhofer, 2016). As the largest tendon in the body, the Achilles tendon is the most common location of xanthomas (Soslowky and Fryhofer, 2016). Using biomechanical data collected during walking on an instrumented treadmill, stiffness of the Achilles tendon was lower

in adults with familial hypercholesterolaemia diagnosed with tendon xanthoma (Squier et al., 2021). Similar findings were observed using a dynamic elastic imaging technique, known as shear wave elastography, where the shear modulus (a proxy for tissue stiffness) was lower and the thickness of the Achilles tendon higher in people with familial hypercholesterolaemia who had very high levels of LDL cholesterol (Zhang et al., 2018)). In contrast, increased stiffness of the patellar tendon was observed in young-middle aged adults with mildly elevated LDL cholesterol (Torgutalp et al., 2020). Differences in findings may be because participants were excluded if they were taking cholesterol-lowering medication, smokers and participants with diabetes, rheumatological or musculoskeletal conditions. Alternatively, differences in tendon sites may influence findings (Coombes et al., 2018), therefore study of the Achilles tendon is warranted.

While the above evidence suggests hypercholesterolaemia may alter tendon mechanical properties, the effects of hypercholesterolaemia, as well as the effects of ageing, on muscle mechanical properties is sparse (Lindemann et al., 2020). It is plausible that alterations in tendon stiffness may affect how muscle deforms when it lengthens (Herbert et al.,

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2019) or influence fascicle operating lengths (Lindemann et al., 2020). As pennate muscles, such as the gastrocnemius, display changes in pennation angle that accompany elongation of muscle fascicles (Herbert et al., 2019), measurement of muscle stiffness and muscle architecture during passive lengthening of a relaxed muscle may provide greater insight into the integrated effects of hypercholesterolaemia on the musculoskeletal system.

The aims of this case-control study were to determine the effects of mild, untreated hypercholesterolaemia on stiffness of the Achilles tendon and gastrocnemius medialis (GM) muscle and on other measures of muscle architecture. To achieve this aim, shear wave elastography was used to estimate the shear wave velocity (SWV) of propagating waves within tendon and muscle providing a proxy for tissue stiffness (Bercoff et al., 2004, Zhang and Fu, 2013). As both active and passive tension are known to increase SWV and influence muscle architecture (Hug et al., 2013, Helfenstein-Didier et al., 2016), we performed measurement in a relaxed state under varying degrees of passive tension on the triceps surae.

2. Methods

2.1. Participants

Participants were recruited between April 2022 – May 2022 via Facebook local community groups and flyers at local pharmacies. Twelve participants (six with hypercholesterolaemia and six control participants) met the selection criteria. All participants were adults aged 50 years and over without a history of cholesterol-lowering medication. Participants were excluded if they required a mobility aid, had experienced lower limb pain or injury requiring treatment or time off work in the last 6 months, previous Achilles rupture, surgery or injections to the Achilles tendon, diabetes diagnosed by a doctor or any systemic, neurological or rheumatological disease or current cancer treatment. Descriptive information about the participants is reported in Table 1.

2.2. Experimental procedure

The study was conducted in accordance with the Declaration of Helsinki and was approved by The Griffith University Human Research Ethics Committee (GU Ref No: 2021/738). Participants attended a single testing session at the Musculoskeletal Health and Persistent Pain laboratory at Griffith University. The experimental procedure is illustrated in Fig. 1. Participants provided written informed consent, underwent fasting blood analysis, pre-experimental preparations and pre-conditioning, prior to assessment of the left Achilles tendon and GM muscle under four different passive loads. Potentially confounding factors including morphology of the Achilles tendon and GM muscle, anthropometric measures, self-reported physical activity and muscular endurance were also evaluated.

2.2.1. Blood analysis and group allocation

Participants were required to not consume food or drinks for 12 h preceding testing of fasting lipids and glycaemic control. Total cholesterol, LDL, HDL and triglycerides, and glycaemic control (HbA1c) were measured from a finger prick blood sample using a point of care device (Afinion AS100, Abbott). Based on Adult Treatment Panel III for classification LDL cholesterol levels, participants were divided into those with elevated LDL (>3.33 mmol/L) or optimal LDL (<2.56 mmol/L) cholesterol (Expert Panel on Detection and Treatment of High Blood Cholesterol in, 2001). Two participants were excluded as LDL values were between these ranges. After analysis was completed, participants were provided with juice and a muesli bar.

2.2.2. Electromyography monitoring of muscle activity

Surface electromyography (EMG) (Trigno Research+, Delsys Inc., Massachusetts,

Table 1

Demographic characteristics, fasting blood measures and morphology of the Achilles tendon and gastrocnemius medialis muscle for participants with mild hypercholesterolaemia (HC) and a control group (CON) with optimal low-density lipoprotein (LDL) cholesterol. Data is summarised as counts (%), mean \pm standard deviation (SD) or median [25th, 75th percentile]. MVPA – moderate to vigorous activity, where time spent on vigorous activity is multiplied by 2; HDL: high-density lipoprotein; HbA1c: glycated haemoglobin; CSA: cross sectional area. Significant p-values (<0.05) are in bold.

	CON (n = 6)	HC (n = 6)	P-value
Demographic characteristics			
Male (n)	3 (50 %)	2 (33.3 %)	0.558
Female (n)	3 (50 %)	4 (66.7 %)	
Age (years)	60.8 \pm 8.6	65.3 \pm 7.3	0.354
Fasting blood analysis			
LDL (mmol/L)	2.1 \pm 0.4	3.6 \pm 0.2	<0.001
HDL (mmol/L)	1.8 \pm 0.5	1.9 \pm 0.6	0.699
Total Cholesterol (mmol/L)	4.2 \pm 0.9	6.2 \pm 0.6	0.001
Triglycerides (mmol/L)	0.8[0.8,1.0]	1.5[1.0,1.9]	0.092
HbA1c (%)	5.3 \pm 0.2	5.5 \pm 0.2	0.214
Anthropometrics			
Height (cm)	172.6 \pm 8.2	163.3 \pm 10.9	0.125
Weight (kg)	78.4 \pm 9.3	73.2 \pm 23.0	0.614
Body mass index (kg/m ²)	26.2 \pm 2.8	26.9 \pm 5.5	0.781
Waist Circumference (cm)	87.7 \pm 9.7	88.3 \pm 17.4	0.943
Physical activity in previous week			
>150 min MVPA n (%)	2 (33.3 %)	4 (66.7 %)	0.248
\geq 2 strength activities n (%)	0 (0 %)	2 (33.3 %)	0.121
Morphology			
Tendon CSA (cm ²)	0.5 [0.5, 0.6]	0.5 [0.4,0.5]	0.169
Tendon thickness (cm)	0.6 \pm 0.2	0.5 \pm 0.2	0.411
Muscle CSA (cm ²)	11.84 \pm 1.9	10.86 \pm 5.4	0.708
Muscle thickness (cm)	1.84 \pm 0.4	1.8 \pm 0.2	0.916
Heel rise test			
Repetitions to fatigue	15.4 \pm 2.7	15.3 \pm 2.0	0.984
Ankle range of motion (°)	29.6 \pm 7.8	32.9 \pm 15.2	0.667
Work (J)	1533 \pm 701	1608 \pm 1232	0.908

USA; Mini Wave Infinity Wireless EMG's, Cometa, Italy) sampling at 2000 Hz was used to continuously monitor muscle activity within the lateral gastrocnemius and tibialis anterior muscles. If muscle activity was visualised during the experiment, the trial was discarded and repeated.

2.2.3. Electrogoniometry measurement of ankle angle

An electrogoniometer (SG110A, Biometrics Ltd, Virginia, USA) connected to a data acquisition system (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK) was used to continuously record talocrural joint angle. Calibration was performed by asking the participant to adopt three ankle positions in standing, confirmed using a manual goniometer. The plantargrade position was set at 90° with greater angles reflecting greater dorsiflexion. Ankle angles were synchronised with experimental data, and first 10 s of each trial was discarded to minimise movement artifacts associated with adding weights. The average ankle angle was manually extracted for each trial.

2.2.4. Application of passive loads and pre-conditioning

Passive loads were applied to passively tension the triceps-surae via a pulley system which was connected to a customised sandal (Fig. 2). The sandal was adjusted so that load was applied at the level of the first metatarsal head, creating a dorsiflexion moment. Pre-conditioning was first performed by adding a 2 kg weight for 5 min to minimise potential effects of stress-relaxation on SWV (Freitas et al., 2015). Following pre-conditioning, a series of loads were applied in an ascending order, and this process was repeated 8 times (i.e. 8 trials). Loads were applied in the same order (0, 0.5, 1.0, 1.5 kg), waiting 10 s for movement artifacts to settle before commencing elastography recording. Elastography recordings of the Achilles tendon and GM muscle were performed in an

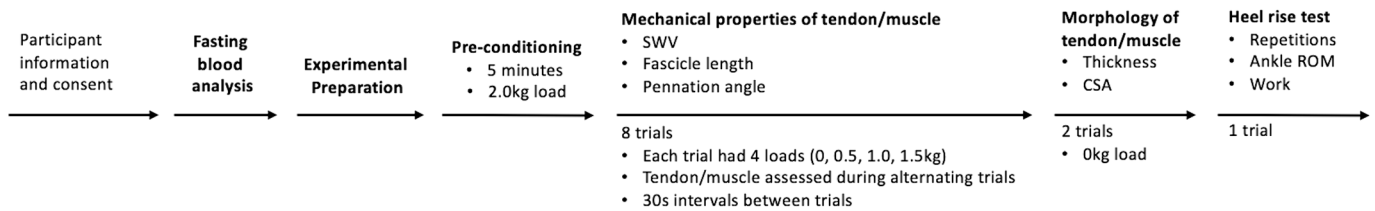


Fig. 1. Experimental procedure. SWV: shear wave velocity (a proxy for tissue stiffness); CSA: Cross-sectional area. ROM: Range of motion.

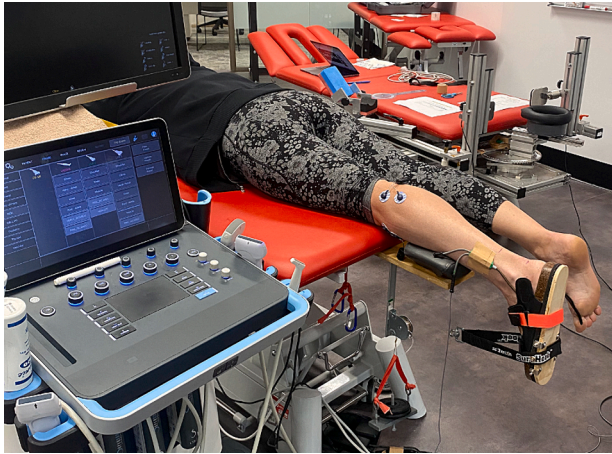


Fig. 2. Experimental set-up. Weights were added to a pulley attached to the forefoot via a sandal. Electrodes were placed over the gastrocnemius lateralis muscle and tibialis anterior muscle (not visible) to monitor muscle activity. Electrogoniometer sensors were aligned with the fibula and 5th metatarsal (not visible) to record ankle angle.

alternating fashion (i.e. trials 1,3,5,7 for tendon; trials 2,4,6,8 for muscle) allowing 30 s intervals between each trial. As the Aixplorer elastography machine had an upper limit of 20 m/s, measurement of tendon SWV beyond 1.5 kg was not possible due to image saturation.

2.3. Experimental outcomes

Experimental outcomes (SWV and muscle architecture) were assessed by a single operator (BKC) with over five years of elastography experience using the Aixplorer Ultrasound Scanner (version 9.3; Supersonic Imagine, Aix-en-Provence, France) with a linear transducer (Mach-30, L18-5 MHz, Vermon, Tours, France). A second researcher applied the loads, documented the time of measurement for synchronisation and monitored muscle activity and angle angles throughout the experiment.

2.3.1. Tendon shear wave velocity (SWV)

SWV was measured over the free Achilles tendon at the midpoint between the calcaneal insertion and soleus muscle-tendon junction with the probe aligned longitudinal to collagen fibres. The 'Musculoskeletal – Foot/Ankle' preset was adopted and the range increased to 1200 kPa (20 m/s). Post processing was performed using the inbuilt measurement tool (Q-box trace) to select a rectangular region of interest measuring approximately 2.5 cm in length and keeping within the peritendinous boundary. Three representative images were each selected from the two 10 s elastography clips recorded for each trial and the average used in analysis. These methods have been adopted in previous studies (Coombes et al., 2018, Coombes et al., 2019).

2.3.2. Muscle shear wave velocity (SWV)

SWV was measured approximately at the midpoint of the GM muscle belly with the probe aligned longitudinal to muscle fascicles. The probe

position was marked on the skin to enable this location to be repeatedly measured. The 'Musculoskeletal – Muscle' preset was adopted and the largest region of interest that avoided aponeurosis and artefacts was selected. Post processing was performed using the largest circle possible (~1cm diameter) within the region of interest using the Q-Box function. Two 10 s elastography clips were recorded for each trial and the average used in analysis. These methods have been adopted in previous studies (Lindemann et al., 2020).

2.3.3. Muscle architecture

Fascicle length and pennation angle were measured from B-mode images overlying elastography maps recorded for each trial. Images were downloaded and Stradwin software (v6.0, Machine Intelligence Laboratory, Cambridge University, UK) used to calculate the straight-line distance between the superficial and deep aponeurosis parallel to the lines of collagenous tissue (fascicle length) and the angle made between the superficial aponeurosis (estimated line of action of the tendon) and the direction of the muscle fascicles (pennation angle). All fascicles were visible in all scans. These methods have been shown to minimize measurement errors (Benard et al., 2009).

2.3.4. Tendon morphology

Tendon morphology was collected as larger tendon thickness can lead to higher SWV due to the effects of guided wave propagation, independent of any change in actual tissue elasticity (Brum et al., 2014, Helfenstein-Didier et al., 2016). Tendon thickness and cross-sectional area (CSA) images were captured at same locations as SWV in the 0 kg condition only. Aixplorer inbuilt software tools were used to measure the maximum perpendicular distance between anterior and posterior tendon boundaries (thickness) and to trace within the peritendon boundaries (CSA) from saved images.

2.3.5. Physical activity and anthropometry

Participants completed items from the Exercise Module of the National Health Survey 2017–18 (ABS, 2018) to assess whether they were sufficiently physically active in the last week (i.e. completing 150 min of moderate to vigorous activity, where time spent on vigorous activity is multiplied by 2) and whether they were meeting the recommended 2 days a week of muscle strengthening activity. (Australian Institute of Health and Welfare, 2003). Waist circumference, height and weight were measured using standardised methods.

2.3.6. Muscle morphology

Muscle morphology was also collected as it is related to the capacity of a muscle to generate force and locomotor performance. These measures were assumed to remain nearly constant during passive lengthening of the triceps muscle tendon unit, hence were measured only in the 0 kg condition (Herbert et al., 2019). GM muscle thickness was captured at the midpoint with the probe aligned longitudinal to the direction of fascicles. Aixplorer in-built software was used to measure the perpendicular distance between the superficial and deep aponeuroses. Muscle CSA was measured using a slow panoramic sweep from medial to lateral to collect sequential axial images and the in-built software used to manually outline the muscle border from the panoramic images. Two images were captured for each measure and average values used in

analysis.

2.3.7. Heel rise test

The heel rise test was performed to fatigue on the left (test) leg for muscular endurance. This test has been shown to detect clinically relevant differences in function between injured and healthy legs in patients with Achilles tendinopathy (Silbernagel et al., 2006, Silbernagel et al., 2010). Participants performed single leg heel lifts at a rate of 1 Hz in sync with a metronome. Participants were asked to keep their knee extended and use their fingertips to lightly maintain balance and standardised encouragement was provided. The number of calf repetitions, average range of motion at the ankle ($^{\circ}$), heel lift height and work (J) were computed using customised scripts on Matlab (R2022a, The MathWorks, Massachusetts, USA). Heel lift height was calculated using foot length and ankle angle ratio during each heel raise repetition. Ankle angle was calculated using the mean of the range achieved across multiple heel raises performed. The number of repetitions was cut off at 90 % of maximum peak angle range, therefore the test was stopped after two consecutive repetitions below 90 %. Work (J) was calculated as the product of participant mass, acceleration due to gravity (9.8 m/s²), heel lift height and repetitions.

3. Reliability

Reliability of SWV, fascicle length, pennation angle and ankle angle were examined by comparing the four consecutive pairs of trials. Average intraclass correlation coefficients (ICCs) from consecutive pairs of trials were computed using an excel spreadsheet (Hopkins, 2000). Standard error of measurement (SEM) and minimal detectable change (MDC) were computed using the following formulae: $SEM = SD \cdot \sqrt{1 - ICC}$ and $MDC = 1.96 \cdot SEM \cdot \sqrt{2}$. ICC values were excellent for SWV, fascicle length and ankle angle, and good for pennation angle (Table 2) (Cicchetti, 1994). As there were no systematic errors, the four trials were averaged for analysis.

As each of the four trials (separated by approximately 2 min) required a new load to be applied and the ultrasound probe to be repositioned, potential sources of variability in tendon SWV due to repeated measurement or loading were explored (Supplementary Table S1). When pairs of consecutive trials were considered, ICC values were similar, suggesting no significant influence of repeated measurement (ICC ranging from 0.85 to 0.87). However, ICC values were highest for the 0 kg condition (ICC 0.9 (0.81, 0.96)) and progressively lower for each 0.5 kg added, although were still excellent for the highest load of 1.5 kg (ICC 0.80 (0.61, 0.90)). Finally, as selection of regions of interest when processing elastography images may also produce variability in tendon SWV, the intra-rater reliability of image processing was determined using data extracted from the same elastography clips by the same assessor on two occasions, 2 weeks apart (Supplementary Table S1). ICCs showed near perfect reliability for image processing (ICCs ≥ 0.98).

Table 2

Reliability estimates for shear wave velocity (SWV), fascicle length, pennation angle and ankle angle. Intraclass correlation coefficients (ICC), standard error of measurement (SEM) and minimal detectable change (MDC) were calculated to examine the reliability of measurement/image capture. Data represents the average of the four consecutive pairs of trials under loads from 0 to 1.5 kg.

	ICC (95 % CI)	SEM (m/s)	MDC (m/s)
Tendon SWV	0.86 (0.80, 0.90)	0.75 m/s	2.07 m/s
Muscle SWV	0.94 (0.92, 0.96)	0.15 m/s	0.41 m/s
Fascicle length	0.87 (0.82, 0.91)	2.13 mm	5.90 mm
Pennation angle	0.63 (0.57, 0.70)	1.22 $^{\circ}$	3.37 $^{\circ}$
Ankle angle	0.97 (0.95, 0.98)	0.92 $^{\circ}$	2.54 $^{\circ}$

3.1. Statistical analysis

Statistical analysis was performed using Stata (Version 13.1, Stata-Corp, Texas). Data was checked for missing data, errors and normality by visualisation and Shapiro Wilk test. Ankle angles were missing for one participant due to electrogoniometer malfunction. Differences in demographic characteristics and morphology were compared between groups using independent unpaired two-tailed t-tests (if normally distributed), Kruskal-Wallis tests (if not normally distributed) or Chi-square tests (for categorical variables). SWV, fascicle length, pennation angle and ankle angle were each analysed using repeated measure analysis of variance (ANOVA), including group (hypercholesterolaemia, control) as a between-subject factor and load (0, 0.5, 1.0, 1.5 kg) as a repeated variable. Differences between groups were reported as mean and 95 % confidence intervals (CI). Where significant group by load interaction was observed, the effect of load was reported for each group separately. Significance was set at $P < 0.05$.

4. Results

4.1. Participants

Participant characteristics and comparisons between the two groups are summarised in Table 1. As expected, LDL and total cholesterol were higher in the hypercholesterolaemia group ($p < 0.01$). There were no differences in HDL cholesterol ($p = 0.699$), triglycerides ($p = 0.092$) or HbA1c ($p = 0.214$). Potential confounding factors including demographic characteristics, anthropometric measures, physical activity and morphology were also similar between groups ($P > 0.05$). Both groups achieved 15 repetitions of the heel rise test before fatigue, with no differences in ankle range of motion or work achieved.

4.2. Tendon and muscle SWV

For tendon SWV (Fig. 3A), there was a significant effect of group ($P = 0.024$). Tendon SWV was 2.4 m/s higher (95 % CI 0.9, 4.0) in participants with hypercholesterolaemia than control participants. This difference exceeded the SEM and MDC for repeated measures. There was a significant effect of load ($P < 0.001$) but no group-by-load interaction, where tendon SWV increased linearly in both groups by 0.8 m/s (95 % CI 0.7, 1.0) for each 0.5 kg added (Table 3). For muscle SWV (Fig. 3B), there were no significant effects of group or group-by-load interaction. As expected, there was a significant effect of load ($P < 0.001$), with SWV increasing linearly in both groups by 0.2 m/s (95 % CI 0.1, 0.2) for each 0.5 kg added (Table 3).

4.3. Muscle fascicle length and pennation angle

For fascicle length (Fig. 3C), there were no significant effects of group or group-by-load interaction. As expected, there was a significant effect of load ($P < 0.001$) with fascicle lengths increasing linearly in both groups by 1.3 mm (95 % CI 1.1, 1.6) for each 0.5 kg added (Table 3). For pennation angle (Fig. 3D), there was no main effect of group ($P = 0.176$), however there was a significant group-by-load interaction ($P = 0.024$), in addition to an effect of load ($P < 0.001$). Pennation angle decreased by 1.1 $^{\circ}$ (95 % CI 1.0, 1.2) for participants with hypercholesterolaemia and by 0.9 $^{\circ}$ (95 % CI 0.8, 1.0) for control participants for each 0.5 kg added (Table 3).

4.4. Ankle angle

For ankle angle (Fig. 3E), there were no effects of group or group-by-load interaction. As expected, there was a significant effect of load ($P < 0.001$), with ankle angle increasing by 1.7 $^{\circ}$ (95 % CI 1.4, 2.1) for each 0.5 kg added (Table 3).

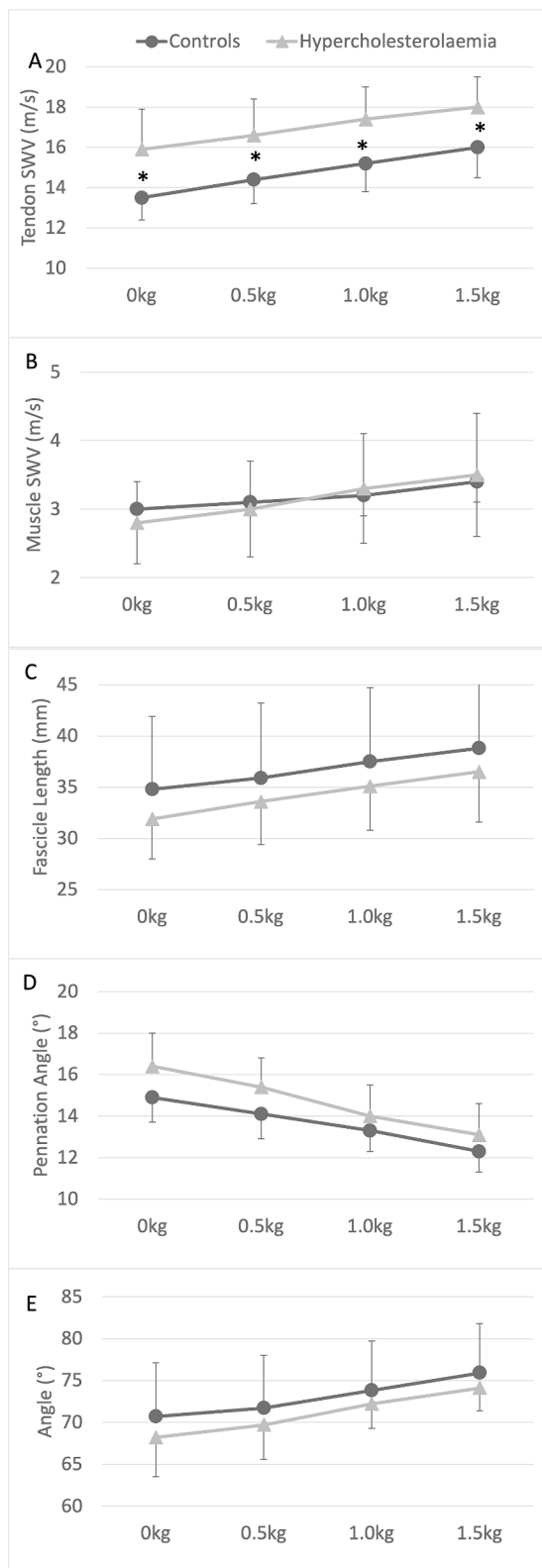


Fig. 3. The effects of increasing load on mean SWV of the Achilles tendon (A), SWV of the GM muscle (B), fascicle length (C), pennation angle (D) and ankle angle (E) for participants with mild hypercholesterolaemia (HC) and a control group (CON) with optimal low-density lipoprotein (LDL) cholesterol. Error bars represent SD. Group differences are highlighted with an asterick. Effects of load were observed for all outcomes (not highlighted).

Table 3

Tendon and muscle shear wave velocity (SWV), fascicle length, pennation angle and ankle angle measured at each passive load for participants with mild hypercholesterolaemia (HC) and a control group (CON) with optimal low-density lipoprotein cholesterol. Data represents mean±SD.

	Group	Load				Effects
		0 kg	0.5 kg	1.0 kg	1.5 kg	
SWV Tendon (m/s)	CON	13.5 ±1.1	14.4 ±1.2	15.2 ±1.4	16.0 ±1.5	Group P = 0.024
	HC	15.9 ±2.0	16.6 ±1.8	17.4 ±1.6	18.0 ±1.5	Load P < 0.001 Group*Load P = 0.768
SWV Muscle (m/s)	CON	3.0 ±0.2	3.1 ±0.2	3.2 ±0.3	3.4 ±0.3	Group P = 0.947
	HC	2.8 ±0.6	3.0 ±0.7	3.3 ±0.8	3.5 ±0.9	Load P < 0.001 Group*Load P = 0.328
Fascicle Length (mm)	CON	34.8 ±7.1	35.9 ±7.3	37.5 ±7.2	38.8 ±7.3	Group P = 0.489
	HC	31.9 ±3.9	33.6 ±4.2	35.1 ±4.3	36.5 ±4.9	Load P < 0.001 Group*Load P = 0.601
Pennation Angle (°)	CON	14.9 ±1.2	14.1 ±1.2	13.3 ±1.0	12.3 ±1.0	Group P = 0.176
	HC	16.4 ±1.6	15.4 ±1.4	14.0 ±1.5	13.1 ±1.5	Load P < 0.001 Group*Load P = 0.024
Ankle Angle (°)	CON	70.7 ±6.4	71.7 ±6.3	73.8 ±5.9	75.9 ±5.9	Group P = 0.529
	HC	68.2 ±4.7	69.7 ±4.1	72.2 ±2.9	74.1 ±2.7	Load P < 0.001 Group*Load P = 0.715

5. Discussion

This study demonstrated that mild hypercholesterolaemia had a significant effect on the mechanical properties of tendon but not of skeletal muscle. Tendon SWV was 2.4 m/s greater in participants with ‘borderline high’ LDL compared to a control group with optimal LDL cholesterol levels. These differences exceeded the MDC values (2.07 m/s) for repeated measurement and were observed across all passive loads. Other measures of muscle architecture were not different between groups, suggesting that alterations in tendon stiffness did not affect how muscle deforms when it lengthens. Furthermore, the two groups showed similar performance on a calf muscle endurance test, suggesting alteration in tendon mechanical properties can occur in the absence of dynamic or functional impairment.

Our findings of greater tendon stiffness but no changes in muscle stiffness or tendon morphology are consistent with a recent elastography study of the patellar tendon and rectus femoris muscle, where higher tendon SWV (and no differences in muscle SWV) was observed in young-middle aged adults with borderline high LDL (≥3.36 mmol/L) compared to a control group (LDL cholesterol ≤ 3.36 mmol/L) (Torgutalp et al., 2020). As both studies excluded participants with musculoskeletal symptoms, it is speculated that changes in SWV may precede changes in tendon morphology and represent subclinical adaptation. In contrast, increased tendon thickness and reduced stiffness are observed in most studies of familial hypercholesterolaemia (Ebeling et al., 1992; Junyent et al., 2005) and most studies in participants with tendinopathy (Arya and Kulig, 2010; Obst et al., 2018). It has been proposed that reduced tendon stiffness may be the result of hyperlipidaemia-driven immune and oxidative stress responses that lead to degeneration of the tendon matrix and increased risk of injury (Fang et al., 2023).

The pathological mechanisms linking hypercholesterolaemia to increased tendon stiffness are less clear. It has been proposed that increased proteoglycan and glycosaminoglycans (GAG) may increase tendon stiffness due to their high affinity for lipids (Waugh et al., 2023). Using a fatigue injury model in rats, increased tendon GAGs were observed in the weeks following fatigue injury, while enzymatic removal

of GAGs from fatigued tendons decreased the dynamic modulus in the toe region (Muljadi and Andarawis-Puri, 2023). The authors speculated that GAG-dependent mechanical changes might help protect the tendon from loading after fatigue injury. Others have proposed that the decline in proteoglycans and GAGs with age might contribute to the age dependent loss in tendon stiffness (Kannus, 2000). Extracellular accumulation of lipids within tendon has been documented from the fourth decade (Kannus et al., 2005), and low tissue turnover of adult tendon (Heinemeier et al., 2013) may explain the isolated effects of hypercholesterolaemia on tendon mechanical properties (Kannus et al., 2005).

Previous research concluded that sonography is superior to magnetic resonance imaging for detection of tendon xanthomas, finding discrete hypochoic lesions with loss of normal fibrillar architecture in participants with familial hypercholesterolaemia even in the absence of tendon thickening (Bude et al., 1994). Our findings suggest that shear wave elastography may provide novel information about early adaptations in tendon mechanical properties due to mild hypercholesterolaemia (Torguitalp et al., 2020). A recent validation study found SWV of the Achilles tendon was correlated with tendon stiffness over small strains quantified using ultrasound imaging with dynamometry (Mifsud et al., 2023). Others have concluded that tendon SWV reflects viscous rather than elastic properties of tendon (Gotschi et al., 2023).

The main limitation of this case-control study is the modest sample size and strict eligibility criteria, excluding participants taking cholesterol-lowering medication, or having diabetes or tendinopathy. As participants with higher LDL cholesterol levels are more likely to be treated with cholesterol-lowering medication, the findings from the current study should not be extrapolated to all individuals with hypercholesterolaemia. While other potentially confounding factors including age, BMI, waist circumference, physical activity levels, morphology and muscle function of the calf musculotendinous unit were similar between groups, future studies that incorporate a larger, more diverse cohort will provide additional insights.

Second, while our study identified an association between mild hypercholesterolaemia and increased tendon stiffness, it doesn't establish causation. Additional longitudinal studies could clarify whether treatment of hypercholesterolaemia by statins can reduce tendon stiffness in participants with mild hypercholesterolaemia. Previous studies have demonstrated that statins are effective in decreasing tendon xanthoma size and are associated with lower risk of tendon pathology in people with familial hypercholesterolaemia (Yang et al., 2019).

Third, evaluation of tissue mechanical properties using shear wave elastography is still in its infancy, and it is acknowledged that there are limitations in assessing non-linear viscoelastic or anisotropic materials such as tendon (Brum et al., 2014; Helfenstein-Didier et al., 2016; Gotschi et al., 2023). As SWV is sensitive to changes in both muscle activity and passive lengthening, we assessed mechanical properties in a passive state (confirmed using surface electromyography) after applying incremental loads of 0.5 kg. Ankle angle was used as a proxy for passive lengthening of the muscle-tendon unit. While there were no differences in ankle angles between groups, we did not measure moment arms, hence intra-tendinous force may vary between participants. Future studies may wish to quantify tendon strain and adjust external load to normalise strain between participants. Our methodology was adopted based on recommendations that tendon SWV is valid only over small strains. As saturation was not measured in this study, it is possible that some participants had SWV values exceeding the upper limit when measured under the highest load. Guided wave propagation and shear wave attenuation due to subcutaneous fat can also affect the quantification of SWV (Brum et al., 2014, Helfenstein-Didier et al., 2016). In the current study there were no differences in tendon thickness between groups, although we did not measure the thickness of subcutaneous fat. Notwithstanding these issues, excellent repeatability was observed for measurement of SWV of tendon and muscle across the four consecutive trials. Using this novel method of application of weights via a pulley, ICCs for tendon SWV were progressively lower when measurement was

performed under greater loads, although were still excellent with loads of 1.5 kg. There was minimal influence of repeated trials on ICC estimates, suggesting stress-relaxation did not significantly impact reliability. As all imaging was performed by a single, experienced assessor, inter-observer reliability was not performed.

The implications of elevated LDL cholesterol and increased tendon stiffness on tendon health and functionality require further investigation. The influence of HDL cholesterol should also be considered, as previous studies suggest a protective effect on tendon thickening factor (Wang et al., 2018). More comprehensive evaluation of muscle SWV along with tendon strain during dynamic or functional tasks could offer a clearer understanding of the practical impact of these findings. However, our preliminary findings suggest that isolated alteration in tendon mechanical properties can occur in the absence of clinical impairments during a dynamic heel rise task.

In conclusion, our study found that even mild levels of hypercholesterolaemia were associated with altered mechanical properties of tendon, but not muscle. Increased tendon SWV was found at low levels of passive strain in adults with untreated hypercholesterolaemia compared to those with optimal LDL cholesterol. As participants did not have known tendon pathology and groups showed similar performance on a heel rise task, it is proposed that increased tendon stiffness may represent a subclinical adaptation. Further research is needed to explore the consequences of such findings on risk of fatigue injury and risk of tendinopathy.

CRedit authorship contribution statement

Brooke K Coombes: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Monica Hanna:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kaelah A. Thompson:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Michel W. Coppieters:** Writing – review & editing, Methodology, Conceptualization. **Taylor J.M. Dick:** Writing – review & editing, Visualization, Methodology, Conceptualization. **Ricardo J Andrade:** Writing – review & editing, Visualization, Methodology, Conceptualization. **Lee Barber:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jbiomech.2024.112048>.

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