Orthopedic Research and Reviews

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Protective Causal Effects of CCL19, CCL23, and IL17A on Achilles Tendinitis: Insights from Bidirectional Mendelian Randomization and Metabolite-Mediated Pathway Analysis

Lu Wei[1,2], Wenqiang Wang[1], Xiang Chen[1], Shunan Dong[1], Hongjie Su[1], Puxiang Zhen[1], Xinyu Nie[3], Qikai Hua[1,4]

[1]Department of Bone and Joint Surgery, (Guangxi Diabetic Foot Salvage Engineering Research Center/Research Centre for Regenerative Medicine), The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, People's Republic of China; [2]Department of Orthopedics, Liuzhou Workers' Hospital, Liuzhou, Guangxi, People's Republic of China; [3]Department of Orthopedics, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, People's Republic of China; [4]Collaborative Innovation Centre of Regenerative Medicine and Medical BioResource Development and Application Co-Constructed by the Province and Ministry, Guangxi Medical University, Nanning, Guangxi, People's Republic of China

Correspondence: Qikai Hua, Email hqk100@yeah.net

Background: Achilles tendinitis (AT) is a prevalent musculoskeletal disorder with unclear etiology. This study aimed to investigate the causal relationships between circulating inflammatory cytokines (ICs), metabolites, and AT risk using bidirectional Mendelian randomization (MR), and to identify potential metabolite-mediated pathways.

Methods: A bidirectional MR design was implemented, integrating genetic instruments for 91 ICs and 1400 metabolites with GWAS summary statistics from the FinnGen consortium. Causal inferences were drawn using inverse variance weighting (IVW), MR-Egger regression, and weighted median approaches, accompanied by sensitivity and mediated analyses.

Results: CCL19, CCL23, and IL17A were identified as protective factors for AT, with CCL23 demonstrating consistent associations across multiple MR methods. 65 metabolite traits were significantly associated with disease risk. Glycochenodeoxycholate glucuronide showed a protective effect (P = 0.002), whereas the alpha-tocopherol to glycerol ratio increased risk (P = 0.011). Mediation analysis indicated six pathways: CCL19 - pantothenate - AT; CCL19 - Picolinate - AT; CCL19 - X-21845- AT; CCL23 - X-12822 - AT; CCL23 - X-18921 - AT; IL17A - cysteinylglycine disulfide - AT.

Conclusion: This is the first MR study to systematically assess the causal roles of ICs and metabolites in AT, identifying CCL19, CCL23, and IL17A as protective factors and highlighting multiple metabolite signatures linked to disease risk, offering novel insights for mechanistic research and targeted intervention.

Keywords: achilles tendinitis, mendelian randomization, inflammatory cytokines, metabolites

Introduction

Achilles tendinitis (AT) is a common overuse injury characterized by localized pain, swelling, and impaired tendon function, which leads to reduced mobility and a diminished quality of life.[1,2] It affects up to 5.9% of athletes and approximately 2.35 per 1000 individuals in the general population.[3] The etiology of AT is complex and mainly related to overuse, mechanical load, improper exercise posture, age, genetic factors, and metabolic diseases (such as diabetes and hyperlipidemia).[4] Repetitive movements, acute injuries, or inappropriate footwear can increase the burden on the Achilles tendon, leading to tissue damage.[5] Additionally, oxidative stress and ICs play a role in the pathological process, affecting the tendon's repair capacity and increasing the risk of inflammation.

Inflammatory cytokines (ICs) are a class of important bioactive substances produced during the body's inflammatory response, playing a key role in tissue injury, repair, and disease progression. In the pathological process of

https://doi.org/10.2147/ORR.S574286
Received: 14 October 2025
Accepted: 5 January 2026
Published: 13 January 2026

tendinitis, multiple ICs are involved, forming a complex regulatory network.[6] Studies have shown that pro-inflammatory ICs such as TNF-α, IL-1β, and IL-6 are elevated in the plasma and local tissues of patients with tendinitis.[7] These ICs promote the release of matrix metalloproteinases, leading to the degradation of collagen fibers and changes in the microenvironment of the tendon. At the same time, anti-inflammatory ICs like IL-10 and TGF-β play an important role in maintaining the inflammatory balance and promoting tissue repair.[8] However, there is still a lack of in-depth understanding of whether there is a direct causal relationship between these ICs and tendinitis, as well as their specific mechanisms of action. Notably, inflammatory signaling is known to reprogram cellular metabolism, suggesting that ICs may influence tendon health not only directly but also through downstream metabolic alterations.

In addition to ICs, the role of metabolites in the pathogenesis of tendinitis has also attracted increasing attention. Metabolomics research has found abnormal changes in various metabolites in the plasma of patients with tendinitis, including alterations in amino acids, lipids, and carbohydrates.[9] In particular, metabolites related to energy metabolism and oxidative stress, such as lactate, pyruvate, and glutathione, may reflect the metabolic state and oxidative stress level of tendon tissue. Moreover, certain metabolites, such as homocysteine, have been shown to directly affect collagen synthesis and the mechanical properties of tendons.[10] However, the causal relationship between these metabolites and tendinitis remains unclear and requires more rigorous research designs to verify. Given the established crosstalk between inflammation and metabolism, we hypothesize that specific metabolites may serve as intermediaries linking ICs to AT risk.

While these molecular insights highlight the biological plausibility of cytokine involvement, the causal nature of these associations remains uncertain due to limitations in existing study designs. Although prior research has underscored the involvement of ICs in the pathophysiology of AT, most findings are derived from observational or cross-sectional studies, thus limiting the ability to infer causality.[11,12] Consequently, there is a pressing need for more rigorous methodological frameworks to clarify these associations. Mendelian randomization (MR) leverages genetic variants as instrumental variables (IVs) to infer causal relationships between exposures and outcomes, minimizing confounding and reverse causality by exploiting the random allocation of alleles during meiosis.[13] The two-sample MR design utilizes summary statistics from separate GWAS for exposure and outcome, enabling large-scale causal inference without individual-level data.[14] A bidirectional MR framework further allows assessment of whether causality operates in one or both directions —particularly relevant here, as inflammation may drive tendon pathology while tendon injury may also trigger inflammatory responses.[15]

In this study, we employed a comprehensive MR framework to test our hypothesis that ICs influence AT risk through both direct and metabolite-mediated pathways. Specifically, we aimed to: (1) investigate the causal effects of circulating ICs on AT risk using bidirectional MR; (2) identify metabolites causally associated with AT; (3) examine whether ICs causally affect these metabolites; and (4) through mediation analysis, evaluate whether specific metabolites serve as intermediaries in the causal pathway from ICs to AT. This integrated approach will provide mechanistic insights into the inflammatory-metabolic axis in tendon pathology and identify potential therapeutic targets.

Methods

Study Design and Analytical Framework

A bidirectional MR design was employed to investigate the causal relationship between circulating ICs and AT. Both forward and reverse MR analyses were conducted, treating cytokines and tendinitis alternately as exposures and outcomes to evaluate their reciprocal effects (Figure 1). Subsequently, MR analysis was extended to assess the causal influence of circulating metabolites on AT, and metabolites with significant associations were identified. Based on these results, potential mechanistic pathways linking ICs, metabolites, and tendinitis were constructed. Mediation analysis was then performed to estimate the indirect effects of metabolites in the causal pathway from cytokines to tendinopathy, aiming to elucidate underlying pathogenic mechanisms.

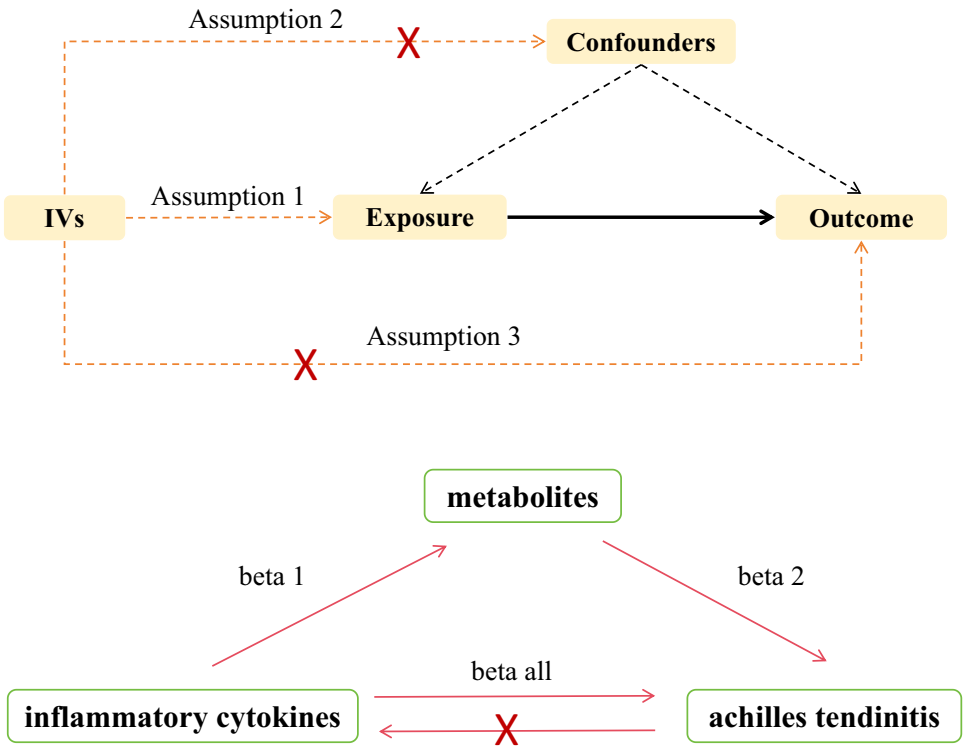

Figure 1 Study design for two-sample MR analysis. This flowchart illustrates the analytical framework of the bidirectional MR study. Arrows indicate the direction of causal inference. The red cross symbol (X) denotes the exclusion of invalid IVs that did not meet the selection criteria. Beta 1 represents the causal effect of ICs on metabolites; Beta 2 represents the causal effect of metabolites on AT; Beta all represents the total causal effect of ICs on AT.

Data Sources and Study Populations

The ICs data was obtained from a large-scale proteomic quantitative trait locus (pQTL) study published in 2023, in which 91 plasma inflammation-related proteins were measured in 14,824 individuals of European ancestry using the Olink Target Inflammation platform.[16] The corresponding GWAS summary statistics are publicly available via the GWAS Catalog (accession numbers GCST90274758 to GCST90274848).

Metabolites data were derived from a genome-wide association study conducted as part of the Canadian Longitudinal Study on Aging (published in 2023), which included 8299 participants of European ancestry. A total of 1091 plasma metabolites and 309 metabolite ratios were quantified using the Metabolon HD4 platform, with batch normalization performed to ensure consistency. The relevant GWAS data are accessible via the GWAS Catalog (accession numbers GCST90199621 to GCST90201020).

Data on AT was extracted from Release 10 (R10) of the FinnGen project, which comprised 3434 confirmed cases and 294,770 controls. FinnGen is a large-scale biomedical initiative that integrates genomic data with electronic health records from Finnish biobanks to explore the genetic architecture of diseases. The GWAS summary statistics for AT can be downloaded from: (gs://finngen-public-data-r10/summary_stats/finngen_R10_M13_ACHILLESTEND.gz).

IVs Selection and Assumption Validation

IVs were selected and evaluated based on the three core assumptions underlying MR: relevance, independence, and exclusion restriction.

First, the relevance assumption requires that IVs be strongly associated with the exposure. Single nucleotide polymorphisms (SNPs) reaching genome-wide significance ($P < 5 \times 10^{-8}$) were selected, and their strength assessed by calculating the F-statistic using the formula $F = R^2 \times (N - 2) / (1 - R^2)$, where R^2 represents the proportion of variance explained and N is the sample size. SNPs with $F > 10$ were considered sufficiently strong instruments.

Second, the independence assumption stipulates that IVs must not be associated with confounders. To address this, principal component analysis was used to adjust for population stratification, and ancestral matching was conducted to ensure genetic homogeneity. Linkage disequilibrium (LD) pruning was performed using a threshold of $r^2 < 0.001$ within a ±250 kb window to retain only the most significant SNPs. Potential confounders such as age, sex, and lifestyle factors were further addressed by stratification or covariate adjustment when necessary.

Third, the exclusion restriction assumption requires that IVs influence the outcome solely through the exposure, with no alternative pathways (i.e., horizontal pleiotropy). Bioinformatic databases such as the GWAS Catalog and PhenoScanner were consulted to identify pleiotropic associations, and functional annotations were performed to assess potential biological relevance. Statistically, we applied five robust MR methods—IVW, MR-Egger regression, weighted median, weighted mode, and simple mode—for sensitivity analyses. MR-Egger intercept testing was conducted to detect unbalanced pleiotropy, Cochran's Q statistics assessed heterogeneity, MR-PRESSO identified and corrected outliers, and leave-one-out analysis evaluated the influence of individual SNPs.

Among these, the IVW method was adopted as the primary analytical approach due to its high statistical efficiency and ease of implementation. Although its assumptions are relatively strict, IVW performs well under conditions of strong instruments and minimal pleiotropy. Therefore, IVW was used for primary estimation, supplemented by alternative MR methods to validate the robustness of the results.

Statistical Analysis and Mediated Effect Assessment

All MR analyses were performed using R software (version 4.3.2), with the "TwoSampleMR" and "gwasglue" packages used to conduct bidirectional two-sample MR analyses. The causal effects of 91 ICs and approximately 1400 metabolites on the risk of AT were assessed. The random-effects IVW method was adopted as the primary analytical approach, with effect estimates reported as odds ratios (ORs) and 95% confidence intervals (CIs). To ensure robustness, multiple sensitivity analyses were conducted, including MR-Egger regression, weighted median estimation, MR-PRESSO outlier test, heterogeneity assessment, and leave-one-out analysis.

Mediation analysis was performed using a three-stage regression framework. First, the total effect of each cytokine on AT (β_all) was estimated. Second, the effects of cytokines on metabolites (β_1) and of metabolites on tendinopathy (β_2) were assessed. The indirect effect was calculated as $\beta_1 \times \beta_2$, and the direct effect as $\beta\_all - \beta_1 \times \beta_2$. The proportion mediated was calculated as $(\beta_1 \times \beta_2)/\beta\_all$. The "MendelianRandomization" package was used to conduct mediation analysis, and the significance of indirect effects was evaluated using either the Sobel test or bootstrap methods.

Results

IVs Selection

We identified 2726 SNPs for ICs (Table S1), 240 SNPs for AT (Table S2), and out of 1400 metabolites, 106 metabolites did not have suitable SNPs identified. The remaining 1294 metabolites had 32,015 SNPs identified (Table S3).

Causal Effects of ICs on AT

We first assessed the causal effects of 91 circulating ICs on AT and identified three candidates with suggestive associations: CCL19, CCL23, and IL17A (Figure 2). Both CCL19 (OR = 0.847, 95% CI: 0.731–0.981, P = 0.026) and IL17A (OR = 0.782, 95% CI: 0.625–0.978, P = 0.032) were inversely associated with disease risk based on the IVW method, indicating potential protective roles. CCL23 demonstrated consistent statistical significance across several MR methods (weighted median, IVW, and simple mode), all with ORs < 1. Although CCL19 and IL17A did not achieve significance across all approaches, they were considered relevant based on the primary IVW analysis, warranting cautious interpretation.

Forest plots (Figure S1A,D,G) showed that most SNPs had confidence intervals crossing the null, suggesting limited individual-level associations. Funnel plots (Figure S1B, E, H) were generally symmetrical, indicating minimal directional bias, except for Figure S1E, which showed slight asymmetry potentially due to horizontal pleiotropy. Scatter plots

exposure	outcome	nsnp	method	pval	OR(95% CI)
C−C motif chemokine 19 levels	Achilles tendinitis	27	MR Egger	0.057	0.750 (0.566 to 0.995)
		27	Weighted median	0.364	0.902 (0.721 to 1.128)
		27	Inverse variance weighted	**0.026**	0.847 (0.731 to 0.981)
		27	Simple mode	0.762	0.948 (0.675 to 1.332)
		27	Weighted mode	0.403	0.904 (0.717 to 1.141)
C−C motif chemokine 23 levels	Achilles tendinitis	25	MR Egger	0.215	0.895 (0.754 to 1.062)
		25	Weighted median	**0.047**	0.864 (0.747 to 0.998)
		25	Inverse variance weighted	**0.040**	0.882 (0.783 to 0.994)
		25	Simple mode	**0.026**	0.690 (0.507 to 0.938)
		25	Weighted mode	0.057	0.854 (0.731 to 0.997)
Interleukin−17A levels	Achilles tendinitis	15	MR Egger	0.536	0.838 (0.485 to 1.446)
		15	Weighted median	0.311	0.861 (0.644 to 1.150)
		15	Inverse variance weighted	**0.032**	0.782 (0.625 to 0.978)
		15	Simple mode	0.643	0.887 (0.539 to 1.458)
		15	Weighted mode	0.602	0.873 (0.531 to 1.435)

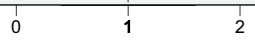

Figure 2 Evaluation of the association between ICs and AT using five MR analysis methods. Forest plots display ORs with 95% CIs for the causal effects of inflammatory cytokines on AT. Five MR methods were applied: inverse variance weighted (IVW), MR-Egger, weighted median, simple mode, and weighted mode. Nsnp means the number of SNP used as IVs. The vertical dashed line represents OR = 1 (null effect); values < 1 suggest protective effects, while values > 1 suggest risk-increasing effects. Error bars represent 95% CIs.

(Figure S1C, F, I) revealed negative slopes between exposures and outcome, supporting the protective effects of the three cytokines.

MR-PRESSO and MR-Egger analyses were used to evaluate pleiotropy (Table S4). Although outlier tests for CCL19 and IL17A returned $P < 0.05$, the corresponding global test P-values (0.767 and 0.788) exceeded 0.05, indicating acceptable levels of bias. MR-Egger intercepts were nonsignificant, suggesting no directional pleiotropy. Heterogeneity tests via Cochran's Q (Table S5) returned $P > 0.05$ across both IVW and MR-Egger models for all three cytokines, suggesting low heterogeneity. Leave-one-out analysis (Figure S2) demonstrated that no single SNP disproportionately influenced the causal estimates, affirming the robustness of the results.

Reverse MR: AT and ICs

Based on the previously identified protective cytokines (CCL19, CCL23, IL17A), a reverse MR analysis was conducted to evaluate whether AT causally affects their circulating levels (Figure 3). Among the three, only IL17A reached statistical significance under the weighted median method (OR = 1.075, 95% CI: 1.013–1.141, P = 0.018), suggesting a modest positive influence of tendinopathy on IL17A. CCL19 and CCL23 showed no significant associations across all five MR methods, with 95% CIs for their ORs including 1, indicating no causal relationship.

In the forest plots (Figure S3A, D, G), most SNPs' effect estimates crossed the null line. Funnel plots (Figure S3B, E) demonstrated symmetrical scatter, indicating no major directional bias; however, Figure S3H showed asymmetry, suggesting possible pleiotropy. Scatter plots (Figure S3C, F, I) revealed a noticeable positive slope only for IL17A, supporting the hypothesis of reverse influence. Horizontal pleiotropy and heterogeneity were assessed using MR-Egger intercepts and Cochran's Q statistics (Tables S4 and S5). Both CCL19 and CCL23 showed $P > 0.05$ in Q tests, suggesting no heterogeneity. In contrast, IL17A exhibited significant heterogeneity ($P < 0.05$), indicating potential bias. Nonetheless, sensitivity analyses supported that such bias was within acceptable limits. Leave-one-out analyses demonstrated consistent effect estimates, indicating that no single SNP dominated the results and that the findings are stable and reliable (Figure S4).

Causal Effects of Circulating Metabolites on AT

MR analysis identified 65 metabolites and metabolite ratios with significant causal associations with AT risk ($P < 0.05$), including 34 risk factors (OR > 1) and 31 protective factors (OR < 1). The complete results are presented in Table S6.

https://doi.org/10.2147/ORR.S574286

exposure	outcome	nsnp	method	pval	OR(95% CI)
Achilles tendinitis	C−C motif chemokine 19	25	MR Egger	0.677	1.015 (0.946 to 1.090)
		25	Weighted median	0.630	0.988 (0.939 to 1.039)
		25	Inverse variance weighted	0.638	1.009 (0.972 to 1.047)
		25	Simple mode	0.939	1.003 (0.919 to 1.095)
		25	Weighted mode	0.836	0.993 (0.927 to 1.063)
Achilles tendinitis	C−C motif chemokine 23	25	MR Egger	0.900	0.995 (0.919 to 1.077)
		25	Weighted median	0.901	1.003 (0.953 to 1.057)
		25	Inverse variance weighted	0.725	0.993 (0.953 to 1.034)
		25	Simple mode	0.771	0.985 (0.890 to 1.090)
		25	Weighted mode	0.621	0.981 (0.909 to 1.059)
Achilles tendinitis	Interleukin−17A	25	MR Egger	0.148	1.080 (0.976 to 1.195)
		25	Weighted median	**0.018**	1.075 (1.013 to 1.141)
		25	Inverse variance weighted	0.218	1.034 (0.980 to 1.091)
		25	Simple mode	0.097	1.110 (0.986 to 1.250)
		25	Weighted mode	0.152	1.110 (0.967 to 1.276)

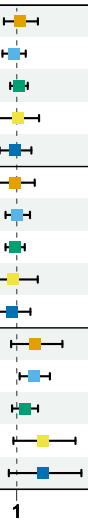

Figure 3 Evaluation of the association between AT and ICs using five MR analysis methods.

Among these, 9 metabolites demonstrated particularly robust associations (Figure 4). The strongest positive association was observed for the alpha-tocopherol to glycerol ratio (OR = 1.270, P = 0.011), indicating a potential risk-enhancing effect. In contrast, the glycerol to palmitoylcarnitine (C16) ratio showed the strongest inverse association (OR = 0.779, P = 0.004), suggesting a protective role. Glycochenodeoxycholate glucuronide (1) demonstrated the most statistically significant association (OR = 0.827, P = 0.002).

No evidence of horizontal pleiotropy was detected based on MR-Egger intercepts or MR-PRESSO tests (Table S7). Additionally, Cochran's Q statistics indicated no significant heterogeneity (Table S8).

Causal Effects of ICs on Selected Metabolites

To further explore potential mechanisms, we examined the causal effects of the three identified ICs on 65 selected metabolites (Figure 5). CCL19 was inversely associated with pantothenate (OR = 0.922, P = 0.046), pyroglutamate (OR = 0.905, P = 0.025), and X-21854 (OR = 0.883, P = 0.024), suggesting protective effects. IL17A showed a significant inverse association with cysteinylglycine disulfide (OR = 0.877, P = 0.015). CCL23 was positively associated with X-18921 (OR = 1.073, P = 0.029), while negatively associated with X-12822, as supported by both the IVW method (OR = 0.931, P = 0.032) and MR-Egger (OR = 0.871, P = 0.006). As shown in Figure S5 and Tables S9, S10, no substantial evidence of horizontal pleiotropy or heterogeneity was detected. Leave-one-out analysis further confirmed the stability of the causal estimates (Figure S6).

Mediated Effects of Metabolites Between ICs and AT

A two-step MR framework was employed to evaluate whether selected metabolites mediated the causal relationship between ICs and AT (Table 1). Six statistically significant mediated pathways were identified. The CCL19–pantothenate pathway exhibited a negative indirect effect (−0.0059), with a mediated proportion of 3.55%. In contrast, the CCL19–pyroglutamate (0.0144) and CCL19–X-21845 (0.0151) pathways showed positive indirect effects, with mediated proportions of −8.63% and −9.06%, respectively, indicating suppressive mediation.

Similarly, the CCL23–X-12822 (0.0101, −8.06%) and CCL23–X-18921 (−0.0088, 6.99%) pathways showed significant mediated effects. The IL17A–cysteinylglycine disulfide pathway demonstrated the strongest negative mediated effect (−0.0235), with a mediated proportion of 9.55%. Although the total effects across all pathways were negative, the directions and magnitudes of direct and indirect effects varied, suggesting complex regulatory mechanisms involving inflammatory and metabolic pathways in the pathogenesis of AT.

exposure	outcome	nsnp	method	pval	OR(95% CI)
N−acetylproline	Achilles tendinitis	20	MR Egger	0.137	0.802 (0.607 to 1.059)
		20	Weighted median	0.072	0.843 (0.699 to 1.015)
		20	Inverse variance weighted	**0.009**	0.836 (0.730 to 0.957)
		20	Simple mode	0.375	0.870 (0.644 to 1.175)
		20	Weighted mode	0.157	0.808 (0.608 to 1.073)
Cysteinylglycine disulfide	Achilles tendinitis	18	MR Egger	0.110	1.238 (0.967 to 1.585)
		18	Weighted median	**0.004**	1.247 (1.075 to 1.447)
		18	Inverse variance weighted	**0.008**	1.196 (1.048 to 1.366)
		18	Simple mode	0.078	1.422 (0.984 to 2.055)
		18	Weighted mode	**0.007**	1.270 (1.091 to 1.479)
Margaroylcarnitine (C17)	Achilles tendinitis	25	MR Egger	0.185	1.218 (0.918 to 1.616)
		25	Weighted median	0.067	1.208 (0.987 to 1.478)
		25	Inverse variance weighted	**0.010**	1.202 (1.046 to 1.382)
		25	Simple mode	0.144	1.293 (0.926 to 1.806)
		25	Weighted mode	0.085	1.269 (0.979 to 1.645)
Sphingomyelin (d18:2/23:1)	Achilles tendinitis	24	MR Egger	**0.048**	1.320 (1.018 to 1.711)
		24	Weighted median	0.067	1.203 (0.987 to 1.467)
		24	Inverse variance weighted	**0.009**	1.205 (1.048 to 1.385)
		24	Simple mode	0.424	1.156 (0.815 to 1.640)
		24	Weighted mode	0.247	1.179 (0.898 to 1.548)
4−hydroxyphenylacetate	Achilles tendinitis	22	MR Egger	0.254	1.133 (0.920 to 1.395)
		22	Weighted median	0.110	1.172 (0.965 to 1.423)
		22	Inverse variance weighted	**0.003**	1.210 (1.068 to 1.370)
		22	Simple mode	0.260	1.168 (0.898 to 1.521)
		22	Weighted mode	0.126	1.168 (0.965 to 1.415)
X−11315	Achilles tendinitis	24	MR Egger	0.061	1.218 (1.001 to 1.483)
		24	Weighted median	0.072	1.157 (0.987 to 1.355)
		24	Inverse variance weighted	**0.010**	1.161 (1.036 to 1.300)
		24	Simple mode	0.175	1.203 (0.928 to 1.559)
		24	Weighted mode	0.128	1.172 (0.962 to 1.428)
N−acetyl−aspartyl−glutamate (naag)	Achilles tendinitis	15	MR Egger	0.095	1.125 (0.990 to 1.280)
		15	Weighted median	**0.020**	1.137 (1.020 to 1.268)
		15	Inverse variance weighted	**0.009**	1.140 (1.033 to 1.258)
		15	Simple mode	0.404	1.136 (0.849 to 1.521)
		15	Weighted mode	0.055	1.136 (1.008 to 1.281)
Glycochenodeoxycholate glucuronide (1)	Achilles tendinitis	23	MR Egger	0.063	0.787 (0.620 to 1.000)
		23	Weighted median	0.119	0.869 (0.729 to 1.037)
		23	Inverse variance weighted	**0.002**	0.827 (0.732 to 0.935)
		23	Simple mode	0.836	0.970 (0.727 to 1.293)
		23	Weighted mode	0.140	0.878 (0.743 to 1.037)
Glycerol to palmitoylcarnitine (C16) ratio	Achilles tendinitis	21	MR Egger	0.955	1.019 (0.542 to 1.913)
		21	Weighted median	**0.015**	0.758 (0.607 to 0.947)
		21	Inverse variance weighted	**0.004**	0.779 (0.659 to 0.922)
		21	Simple mode	0.162	0.745 (0.501 to 1.109)
		21	Weighted mode	0.103	0.730 (0.509 to 1.047)

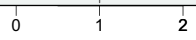

Figure 4 IVW method results of MR analysis for the effect of circulating metabolites on AT.

Discussion

This study is the first to systematically explore the causal relationship between ICs, metabolites, and AT using the MR method, filling a gap in research in this field. Compared to traditional observational studies, the MR method provides more reliable causal inference by avoiding confounding factors and reverse causality interference.

The findings related to CCL19 and CCL23 are partially consistent with some previous studies. Research has shown that these chemokines can promote the recruitment of repair-type macrophages and are involved in tissue repair.[17] As members of the CC chemokine family, both CCL19 and CCL23 play critical roles in regulating immune responses and inflammation balance. CCL23 has been shown to be elevated in patients with systemic sclerosis and associated with

https://doi.org/10.2147/ORR.S574286

exposure	outcome	nsnp	method	pval	OR(95% CI)
C−C motif chemokine 19 levels	Pantothenate levels	34	MR Egger	0.759	0.977 (0.840 to 1.135)
		34	Weighted median	0.156	0.916 (0.811 to 1.034)
		34	Inverse variance weighted	**0.046**	0.922 (0.851 to 0.999)
		34	Simple mode	0.174	0.856 (0.688 to 1.066)
		34	Weighted mode	0.295	0.905 (0.754 to 1.087)
C−C motif chemokine 19 levels	Picolinate levels	34	MR Egger	0.067	0.854 (0.725 to 1.005)
		34	Weighted median	0.253	0.924 (0.808 to 1.058)
		34	Inverse variance weighted	**0.025**	0.905 (0.830 to 0.988)
		34	Simple mode	0.237	0.844 (0.640 to 1.112)
		34	Weighted mode	0.362	0.912 (0.750 to 1.108)
C−C motif chemokine 19 levels	X−21845 levels	34	MR Egger	0.423	0.918 (0.747 to 1.128)
		34	Weighted median	**0.015**	0.818 (0.696 to 0.962)
		34	Inverse variance weighted	**0.024**	0.883 (0.793 to 0.983)
		34	Simple mode	0.232	0.839 (0.633 to 1.113)
		34	Weighted mode	0.133	0.839 (0.671 to 1.049)
C−C motif chemokine 23 levels	X−12822 levels	32	MR Egger	0.416	1.039 (0.948 to 1.139)
		32	Weighted median	0.138	1.065 (0.980 to 1.157)
		32	Inverse variance weighted	**0.029**	1.073 (1.007 to 1.142)
		32	Simple mode	0.246	1.149 (0.913 to 1.447)
		32	Weighted mode	0.115	1.067 (0.987 to 1.154)
C−C motif chemokine 23 levels	X−18921 levels	32	MR Egger	**0.006**	0.871 (0.794 to 0.955)
		32	Weighted median	0.059	0.925 (0.854 to 1.003)
		32	Inverse variance weighted	**0.032**	0.931 (0.872 to 0.994)
		32	Simple mode	0.887	0.985 (0.799 to 1.214)
		32	Weighted mode	0.105	0.930 (0.854 to 1.013)
Interleukin−17A levels	Cysteinylglycine disulfide levels	21	MR Egger	**0.011**	0.724 (0.578 to 0.906)
		21	Weighted median	0.079	0.876 (0.756 to 1.015)
		21	Inverse variance weighted	**0.015**	0.877 (0.789 to 0.975)
		21	Simple mode	0.348	0.876 (0.668 to 1.148)
		21	Weighted mode	0.342	0.870 (0.658 to 1.151)

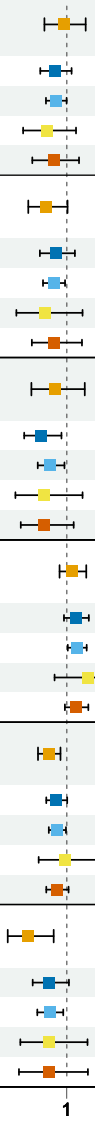

Figure 5 Evaluation of the association between circulating ICs and metabolites using five MR analysis methods.

disease activity,[18] while also serving as a biomarker for brain injury in ischemic stroke.[19] This dual role suggests that its effects may be tissue- and pathology-dependent. In the context of AT, our findings suggest that CCL23 may exert protective effects by promoting healthy tissue remodeling through regulating the balance between matrix metalloproteinases and their inhibitors.[20] Similarly, CCL19, as the primary ligand of CCR7, plays a key role in immune cell migration

Table 1 Mediated Effect of Metabolites in the Influence of ICs on AT

Pathway	Beta 1	Beta 2	Beta All	Mediated Effect	Mediated Proportion (%)
CCL19 - pantothenate -AT	−0.0812	0.0729	−0.1666	−0.0059	3.55
CCL19 - Picolinate - AT	−0.0994	−0.1447	−0.1666	0.0144	−8.63
CCL19 - X-21845- AT	−0.1246	0.1211	−0.1666	0.0151	−9.06
CCL23 - X-12822 - AT	0.0700	0.1440	−0.1252	0.0101	−8.06
CCL23 - X-18921 - AT	−0.0718	0.1220	−0.1252	−0.0088	6.99
IL17A - cysteinylglycine disulfide - AT	−0.1311	0.1217	−0.2461	−0.0235	9.55

and lymphoid organ homeostasis.[21,22] The protective effect of CCL19 in AT may be related to its ability to regulate immune cell infiltration and reduce the inflammatory cascade, thereby facilitating tendon repair. Together, these findings highlight the importance of chemokine-mediated immune regulation in tendon homeostasis.

IL-17 and its related pathways show potential protective effects in AT, which contrasts interestingly with its role as a pro-inflammatory factor in other inflammatory diseases. IL-17, a pleiotropic cytokine, plays an essential role in antimicrobial immune surveillance and maintaining epithelial barrier integrity. IL-17 may offer protective effects in tendon tissue through several mechanisms. First, IL-17 might maintain the structural integrity of the Achilles tendon by enhancing the proliferation and repair capabilities of fibroblasts. A study by Cheng et al indicated that IL-17 can modulate the inflammatory response and promote tissue repair after early tendon injury.[23] Second, IL-17 may strengthen the mechanical strength of the Achilles tendon by promoting the synthesis of extracellular matrix proteins such as collagen and elastin. This aligns with the views of Bisoendial and Lubberts, who emphasized the critical role of IL-17 in tissue repair.[24] Additionally, IL-17 might balance the local inflammatory response by modulating the expression of pro-inflammatory cytokines like IL-1β, preventing excessive inflammation from damaging tendon tissue. This protective effect may be mediated through IL-17 receptor signaling pathways, such as ST2 and IL17RA, whose expression in tendon tissue might be associated with inflammation resolution and tissue repair.[25] It is important to note that the protective role of IL-17 may depend on its concentration and temporal dynamics. In the early stages of tendinitis, moderate IL-17 expression might be beneficial for tissue repair, whereas sustained high levels of IL-17 could turn harmful. This dual effect suggests that the IL-17 pathway could be a potential therapeutic target for AT, requiring precise modulation rather than simple inhibition.

Notably, this study systematically identified 65 metabolites with causal relationships to AT risk through MR analysis. These metabolites can be broadly categorized into several functional groups. First, lipid metabolism-related metabolites showed significant associations: the glycerol to palmitoylcarnitine (C16) ratio was negatively correlated with AT (OR = 0.779, p = 0.004), reflecting the importance of fatty acid β-oxidation and energy metabolism in tendon pathology.[26,27] Carnitine, as an essential cofactor in fatty acid β-oxidation, may reflect changes in energy metabolism reprogramming, which is related to the increased energy demands of tendons under mechanical stress.[28] Second, oxidative stress-related metabolites were prominently featured: the α-tocopherol to glycerol ratio was significantly associated with increased AT risk (OR = 1.270, p = 0.011), revealing the potential role of oxidative stress and antioxidant balance in tendinitis pathology. As an important fat-soluble antioxidant, elevated tocopherol levels may represent a compensatory response to tissue oxidative damage.[29] Our findings support the theory that imbalance between oxidation and antioxidant systems contributes to AT pathogenesis.[30] Third, several unnamed metabolites (such as X-11315, X-12822, etc.) were also identified as causally associated with AT. Although their specific identities and functions remain unclear, they may represent unexplored metabolic pathways, aligning with the theory that tendon degeneration involves complex metabolic network remodeling.[31]

A key contribution of this study is the identification of metabolite-mediated pathways linking inflammatory cytokines to AT risk, directly supporting our initial hypothesis that ICs influence tendon health through downstream metabolic alterations. Through mediation analysis, we identified six significant pathways, with the IL17A–cysteinylglycine disulfide–AT pathway showing the largest mediated proportion (9.55%). Cysteinylglycine disulfide, as an important component of glutathione metabolism, participates in cellular antioxidant defense and may serve as a key intermediary linking IL17A-mediated immune regulation to tendon redox homeostasis.[32] Similarly, the CCL19–pantothenate–AT pathway (mediated proportion 3.55%) implicates coenzyme A biosynthesis and energy metabolism in the protective effects of chemokines. These findings reveal that inflammatory cytokines exert their effects on AT not only through direct immunomodulatory mechanisms but also by reshaping the metabolic microenvironment—a concept we term the "inflammatory-metabolic axis" in tendon pathology. This integrated framework provides novel therapeutic targets that go beyond simple anti-inflammatory approaches.

Given the observational nature of existing tendon studies, the use of MR provides a quasi-experimental framework for causal inference. It enables efficient screening of candidate risk factors using large-scale GWAS datasets. However, MR relies on several assumptions: strong instrument relevance, independence from confounders, and the absence of horizontal pleiotropy.[33] In this study, rigorous selection criteria were applied, and multiple sensitivity analyses—

including MR-Egger regression, weighted median estimation, and MR-PRESSO—were used to address pleiotropy. Nonetheless, MR assumes linear exposure–outcome relationships and may not fully capture gene–environment interactions or tissue-specific effects. These limitations should be considered when interpreting causal estimates.

Several mechanistic pathways may underlie the observed associations. CCL19 and CCL23 may regulate immune balance by promoting recruitment of M2 macrophages and attenuating chronic inflammation. The metabolite findings suggest disruption in lipid metabolism, mitochondrial energy pathways, and redox balance, all of which may influence tendon matrix turnover.[34] Mediated analyses further support the hypothesis that ICs act, at least in part, through specific metabolic intermediates.[35] These biological pathways likely interact to shape the development and progression of tendinopathy.

Taken together, this study provides novel insights into the inflammatory and metabolic underpinnings of AT and highlights several candidate biomarkers and therapeutic targets. However, several limitations merit attention. First, all GWAS datasets were derived from European ancestry populations, which may limit applicability to other ethnic groups. Second, AT cases were defined using electronic health records, lacking clinical phenotyping and differentiation between acute and chronic forms. Third, some associations showed modest effect sizes and were only significant under the IVW method, requiring cautious interpretation. Fourth, MR assumes linearity and cannot fully account for non-linear effects or complex biological interactions. Finally, the absence of functional validation is a key limitation. Further studies incorporating multi-ethnic populations and experimental models are needed to strengthen the evidence base and clarify biological mechanisms.

Conclusion

This study establishes a causal framework linking ICs, metabolites, and AT. CCL19, CCL23, and IL17A were identified as protective factors against AT, and their effects are partially mediated through specific metabolic pathways, including redox metabolism (cysteinylglycine disulfide) and energy metabolism (pantothenate). These findings reveal an inflammatory-metabolic axis in tendon pathology and provide novel targets for mechanistic research and therapeutic intervention.

Data Sharing Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Additional Material.

Ethics Approval and Consent to Participate

This study relied on publicly accessible summary statistics from previously published research and consortia. Each original study involved in this research had received ethical clearance from their respective review boards, and all participants had given informed consent. This study was reviewed by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University and was determined to be exempt from full ethical review approval. The exemption was granted because the study exclusively utilized de-identified, publicly available summary-level data from genome-wide association studies, with no access to individual-level participant data, no direct participant contact, and no collection of new biological samples or personal information.

Author Contributions

Lu Wei contributed to conceptualization, methodology, investigation, data curation, formal analysis, writing the original draft, visualization, and funding acquisition. Wenqiang Wang participated in methodology, investigation, data curation, formal analysis, and writing – review and editing. Xiang Chen, Shunan Dong, Hongjie Su, and Puxiang Zhen were involved in investigation, data collection, formal analysis, and validation. Xinyu Nie provided resources, contributed to methodology and validation, and participated in writing – review and editing. Qikai Hua contributed to conceptualization, methodology, supervision, project administration, and writing – review and editing. All authors have agreed on the journal to which the article has been submitted; reviewed and agreed on all versions of the article before submission,

during revision, the final version accepted for publication, and any significant changes introduced at the proofing stage; and agree to be accountable for all aspects of the work.

Funding

This article is supported by Self-funded Research Project of Guangxi Zhuang Autonomous Region Health Commission (Z-B20231448).

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Kujala UM, Sarna S, Kaprio J. Cumulative incidence of achilles tendon rupture and tendinopathy in male former elite athletes. *Clin J Sport Med*. 2005;15(3):133–135. doi:10.1097/01.jsm.0000165347.55638.23
2. Scott A, Backman LJ, Speed C. Tendinopathy: update on pathophysiology. *J Orthop Sports Phys Ther*. 2015;45(11):833–841. doi:10.2519/jospt.2015.5884
3. Albers IS, Zwerver J, Diercks RL, Dekker JH, Van den Akker-Scheek I. Incidence and prevalence of lower extremity tendinopathy in a Dutch general practice population: a cross sectional study. *BMC Musculoskelet Disord*. 2016;17(16). doi:10.1186/s12891-016-0885-2
4. Agabalyan N. Tendinopathy – from basic science to treatment. *Int J Exp Pathol*. 2013;94(4):A1. doi:10.1111/iep.12034
5. Thorpe CT, Screen HR. Tendon Structure and Composition. *Adv Exp Med Biol*. 2016;920:3–10. doi:10.1007/978-3-319-33943-6_1
6. Dean BJF, Dakin SG, Millar NL, Carr AJ. Review: emerging concepts in the pathogenesis of tendinopathy. *Surgeon*. 2017;15(6):349–354. doi:10.1016/j.surge.2017.05.005
7. Rees JD, Stride M, Scott A. Tendons–time to revisit inflammation. *Br J Sports Med*. 2014;48(21):1553–1557. doi:10.1136/bjsports-2012-091957
8. Staines KA, MacRae VE, Farquharson C. The importance of the SIBLING family of proteins on skeletal mineralisation and bone remodelling. *J Endocrinol*. 2012;214(3):241–255. doi:10.1530/joe-12-0143
9. Nakama K, Gotoh M, Yamada T, et al. Interleukin-6-induced activation of signal transducer and activator of transcription-3 in ruptured rotator cuff tendon. *J Int Med Res*. 2006;34(6):624–631. doi:10.1177/147323000603400607
10. Fenwick SA, Hazleman BL, Riley GP. The vasculature and its role in the damaged and healing tendon. *Arthritis Res*. 2002;4(4):252–260. doi:10.1186/ar416
11. van Sterkenburg MN, Kerkhoffs GM, Kleipool RP, Niek van Dijk C. The plantaris tendon and a potential role in mid-portion Achilles tendinopathy: an observational anatomical study. *J Anatomy*. 2011;218(3):336–341. doi:10.1111/j.1469-7580.2011.01335.x
12. Bahari M, Taslimipour S, Moazenzadeh A. Different clinical presentations of Achilles tendinopathy: a cross-sectional study. *Rheumatol Int*. 2024;44(12):2991–2995. doi:10.1007/s00296-023-05485-1
13. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. 2018;362:k601. doi:10.1136/bmj.k601
14. Pierce BL, Burgess S. Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators. *Am J Epidemiol*. 2013;178(7):1177–1184. doi:10.1093/aje/kwt084
15. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet*. 2014;23(R1):R89–98. doi:10.1093/hmg/ddu328
16. Zhao JH, Stacey D, Eriksson N, et al. Genetics of circulating inflammatory proteins identifies drivers of immune-mediated disease risk and therapeutic targets. *Nat Immunol*. 2023;24(9):1540–1551. doi:10.1038/s41590-023-01588-w
17. Yousaf H, Khan MIU, Ali I, Munir MU, Lee KY. Emerging role of macrophages in non-infectious diseases: an update. *Biomed Pharmacother*. 2023;161(114426). doi:10.1016/j.biopha.2023.114426
18. Yanaba K, Yoshizaki A, Muroi E, et al. Serum CCL23 levels are increased in patients with systemic sclerosis. *Arch Dermatol Res*. 2011;303(1):29–34. doi:10.1007/s00403-010-1078-8
19. Bonaventura A, Montecucco F. CCL23 is a promising biomarker of injury in patients with ischaemic stroke. *J Intern Med*. 2018;283(5):476–478. doi:10.1111/joim.12742
20. Kim CS, Kang JH, Cho HR, et al. Potential involvement of CCL23 in atherosclerotic lesion formation/progression by the enhancement of chemotaxis, adhesion molecule expression, and MMP-2 release from monocytes. *Inflamm Res*. 2011;60(9):889–895. doi:10.1007/s00011-011-0350-5
21. Korbecki J, Grochans S, Gutowska I, Barczak K, Baranowska-Bosiacka I. CC chemokines in a tumor: a review of pro-cancer and anti-cancer properties of receptors CCR5, CCR6, CCR7, CCR8, CCR9, and CCR10 ligands. *Int J Mol Sci*. 2020;21. doi:10.3390/ijms21207619.
22. Korbecki J, Kojder K, Simińska D, et al. CC chemokines in a tumor: a review of pro-cancer and anti-cancer properties of the ligands of receptors CCR1, CCR2, CCR3, and CCR4. *Int J Mol Sci*. 2020;21. doi:10.3390/ijms21218412
23. Cheng JH, Jhan SW, Hsu CC, Chiu HW, Hsu SL. Extracorporeal shockwave therapy modulates the expressions of proinflammatory cytokines IL33 and IL17A, and their receptors ST2 and IL17RA, within the articular cartilage in early avascular necrosis of the femoral head in a rat model. *Mediators Inflamm*. 2021;2021:9915877. doi:10.1155/2021/9915877
24. Bisoendial R, Lubberts E. A mechanistic insight into the pathogenic role of interleukin 17A in systemic autoimmune diseases. *Mediators Inflamm*. 2022;2022:6600264. doi:10.1155/2022/6600264
25. Rodríguez-Montaño R, Alarcón-Sánchez MA, Lomelí-Martínez SM, Martínez-Bugarin CH, Heboyan A. Genetic variants of the IL-23/IL-17 axis and its association with periodontal disease: a systematic review. *Immun Inflamm Dis*. 2025;13(2):e70147. doi:10.1002/iid3.70147

26. Abate M, Schiavone C, Salini V, Andia I. Occurrence of tendon pathologies in metabolic disorders. *Rheumatology*. 2013;52(4):599–608. doi:10.1093/rheumatology/kes395
27. Dakin SG, Newton J, Martinez FO, et al. Chronic inflammation is a feature of Achilles tendinopathy and rupture. *Br J Sports Med*. 2018;52 (6):359–367. doi:10.1136/bjsports-2017-098161
28. Riley G. Tendinopathy–from basic science to treatment. *Nat Clin Pract Rheumatol*. 2008;4(2):82–89. doi:10.1038/ncprheum0700
29. Millar NL, Murrell GA, McInnes IB. Inflammatory mechanisms in tendinopathy - towards translation. *Nat Rev Rheumatol*. 2017;13(2):110–122. doi:10.1038/nrrheum.2016.213
30. Wang S, Yao Z, Zhang X, et al. Energy-supporting enzyme-mimic nanoscaffold facilitates tendon regeneration based on a mitochondrial protection and microenvironment remodeling strategy. *Adv Sci*. 2022;9(31):e2202542. doi:10.1002/advs.202202542
31. Steinmann S, Pfeifer CG, Brochhausen C, Docheva D. Spectrum of tendon pathologies: triggers, trails and end-state. *Int J Mol Sci*. 2020;21(3). doi:10.3390/ijms21030844
32. Yao Z, Qian Y, Jin Y, et al. Biomimetic multilayer polycaprolactone/sodium alginate hydrogel scaffolds loaded with melatonin facilitate tendon regeneration. *Carbohydr Polym*. 2022;277:118865. doi:10.1016/j.carbpol.2021.118865
33. Richmond RC, Davey Smith G. Mendelian randomization: concepts and scope. *Cold Spring Harbor Perspectives Med*. 2022;12(1). doi:10.1101/cshperspect.a040501
34. Muoio DM. Metabolic inflexibility: when mitochondrial indecision leads to metabolic gridlock. *Cell*. 2014;159(6):1253–1262. doi:10.1016/j.cell.2014.11.034
35. Kannus P. Structure of the tendon connective tissue. *Scand J Med Sci Sports*. 2000;10(6):312–320. doi:10.1034/j.1600-0838.2000.010006312.x