The aetiology of pain in chronic midportion Achilles tendinopathy

By

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Submitted to the University of Cape Town in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in the Department of Human Biology, Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

AUGUST 2020

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DECLARATION

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PhD THESIS TITLE: THE AETIOLOGY OF PAIN IN CHRONIC MIDPORTION ACHILLES TENDINOPATHY

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I gratefully received guidance and assistance with some of the statistical analyses included in this thesis from Trevor Mafu, and genotyping from Senanile Dlamini as included in the acknowledgments.

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I confirm that my co-authors have agreed (Appendix I) that I may include the following publication(s) in my thesis.

1. **Mkumbuzi, N.S**., September, A.V., Posthumus, M., Oulo, B., Mafu, T.S., & Collins, M. (2020) Characterisation of Achilles tendon pain using multidimensional pain scales. *Journal of Science and Medicine in Sport.* **23(3):** 258-263, DOI 10.1016/j.jsams.2019.10.016
2. **Mkumbuzi, N.S**., Jorgensen, O., Mafu, T.S., September, A.V., Posthumus, M., & Collins, M (2020). Tendon structure is not related to self-reported symptoms in recreational runners with chronic Achilles tendinopathy*. Translational Sports Medicine* **3(6):** 589-598; DOI: 10.1002/tsm2.183.
3. **Mkumbuzi, N.S**., Mafu, T.S., September, A.V., Posthumus, M., & Collins, M (2020). Conditioned pain modulation is not altered in recreational athletes with chronic Achilles tendinopathy. *Translational Sports Medicine* 00: 1-7. DOI: org/10.1002/tsm2.201
4. **Mkumbuzi, N.S**., Dlamini, S.B., Mafu, T.S., Posthumus, M., September, A.V., & Collins, M. A novel evaluation of *COMT, SCN9A, TAC1* and *TACR-1* polymorphisms in a South African cohort of recreational athletes with chronic Achilles tendinopathy highlights an association between *COMT*, pain sensitivity and self-reported tendon pain. ***Under review***

DEDICATION

To my brothers, Emmanuel and Roland Mkumbuzi. I inspire you. You're welcome fellas.

To Vyvienne M'kumbuzi (1973-2016), you would have been proud *Chihera*.

To my grandma, Helena Mkumbuzi, *veni, vidi, vici.*

To my grandpa, Wenceslaus Mkumbuzi, the only person who reads my theses and publications, not because he must but because he wants to.

ACKNOWLEDGEMENTS

Firstly, on behalf of every student, past, present, and future, I would like to thank Larry Tesler (1946-2020). We are forever indebted to you sir.

I would like to express my profound gratitude to my main supervisors, Malcolm, Alison and Mike for their patient guidance in this process. For allowing me room to grow, to think independently and to explore my scientific depths. Thank you for not only advising me through this process but for moulding me into a scientist and modelling for me what it means to be a good researcher and scientist. Additionally, thank you to Prof Michael Kjær, my supervisor during my PhD secondment, for welcoming me into his research group and ensuring that I received adequate training in the techniques needed to conduct my PhD studies.

I would also like to acknowledge financial assistance gratefully received from the National Research Foundation of South Africa (NRF Grantholder bursary), the European Union's Horizon 2020 Research and Innovation Programme under the Marie Sklodowska-Curie grant (RUBICON) and the Margaret McNammara Education Grant.

Additionally, I would also like to thank the athletes who participated in this study, without whom this body of work would still be theoretical and Winelands Radiology for availing their facilities for me to use during testing of the participants.

Thank you to Neezaam Karim, whose office and life has probably been very peaceful since I finished my experiments. To Trevino Larry who has probably never had to answer as many dumb IT questions as he did from me. To Ayesha Hendricks and Lesa Sivewright, evidence that super-heroes don't wear capes, they sit behind a desk and know grant numbers from memory. To Berenice Karelse and Anzio Hantam for their assistance with managing the participants during the testing. To Nareema Behardien and Elsabe Botha, whose knowledge of ultrasound is so extensive, it borders on witchcraft, and Dr Oscar Jørgensen for his assistance with the ultrasound analysis. I would also like to thank Senanile Dlamini, for her assistance with the lab work, and Trevor Mafu and Dr Brenda Oulo for assisting me with the statistical analyses.

My sincere thanks go to my colleagues in the GEMs research group for fostering a supportive, collegial, and inspiring environment at ESSM.

Personally, I would also like to thank (again) Her Royal Highness of eSwatini and soon to be doctor, Senanile Dlamini; peasants, but also soon to be doctors: Trevor Mafu, Siddiqah George, Raffaele Mazzolari and Elisabeth Knittenfelder for being my friends and companions on this journey and always being there when I needed the soul cleansing effects of a long *kvetch*.

To my hiking friends, the Mountain Slayers, a rag tag bunch of misfits and muppets whom I am privileged to call my friends, thank you for the generous doses of mountain and meme therapy.

To my cousin, Prof Vyvienne M'kumbuzi (1973-2016), a great academic who inspired me to be one as well and would have been so proud that I put my grey matter to good use.

To my mentor, Prof Midion Chidzonga, for indulging and supporting my academic (and personal) madness and understanding my vision for myself and encouraging me to take chances.

To Dobbi's mum, Marina Stoinova, for turning what would have been a fast food and sugary snacks fuelled writing endeavour into a well-nourished experience. Thank you for ensuring that at a time when I had to write this thesis and the manuscripts thereof, my next meal, grocery shopping or medication refills were the least of my worries.

To my boyfriend, Dobromir Stoinov (Dobbi), who probably still has no clue what Reviewer 2, echogenicity or TaqMan assays are but made the correct sympathetic noises whenever I started ranting and raving. Thank you for (literally) reminding me to eat and to go outside and feel the sun. *Now* I can join you for the walks on the beach.

To my siblings (some of whom are actually cousins), for always being a pleasant break from reality. To my family in Cape Town: Jean, Herbert, Tanya and Panashe Munyikwa; Nicole, Keith and Sandra Chineka for always checking in on me. The rest of my family who were further away, thank you for the phone calls to check in on me or to gossip about the others. Words cannot adequately express my appreciation for your support.

To my grandmother, Helena Mkumbuzi, who still thinks I studied physics (it was physiotherapy) and has always emphasised the value of tenacity and running one's own race and for never giving up on our education. Which for me was physics, apparently. *Ngiyabonga. eTshuma naCammy.*

To my grandfather, Wenceslaus Mkumbuzi, our family's reader-in-chief and the original *philos sophos* who taught us to love to read for pleasure. Thank you for teaching us to be curious. *eMpofu mkhulu.*

To myself, Nonhlanhla. To the young woman who worked two jobs to fund her PhD, who wrote her thesis in lockdown during a global pandemic while fending off a 16-week spell of COVID-19 symptoms and working part time. To the scientist who is growing into her own and discovering her passion. To Nonhlanhla, thank you for always being there for you and for seeing this through.

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Publications in peer reviewed journals

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Conference presentations

1. Collins, M, **Mkumbuzi, N.S**., Dlamini, S.B., Mafu, T.S., Posthumus, M., & September, A.V., Association between self-reported tendon pain and genetic variants. European College of Sport Science Conference. Seville, Spain, 2020. Oral presentation*****
2. **Mkumbuzi, N.S**., Senoamali, H., September, A.V., & Collins, M. Gait parameters in recreational runners with early stage Achilles tendinopathy. European College of Sport Science Conference. Seville, Spain, 2020. Oral presentation*****
3. **Mkumbuzi, N.S.,** September, A.V., Posthumus, M., Oulo, B., Mafu, T.S., & Collins, M. Characterisation of Achilles tendon pain using multidimensional pain scales. International Scientific Tendinopathy Symposium. Groningen, The Netherlands, 2019. Oral presentation
4. **Mkumbuzi, N.S**., September, A.V., Posthumus, M., Oulo, B., Mafu, T.S., & Collins, M. Characterisation of Achilles tendon pain using multidimensional pain scales. UCT Department of Human Biology, Postgraduate research day. Cape Town, South Africa. 2018. Poster presentation

*****conference postponed due to the COVID-19 pandemic.

LIST OF ABBREVIATIONS

A	adenine
AA	arachidonic acid
ACC	anterior cingulate cortex
ACh	acetylcholine
ACL	anterior cruciate ligament
ALOX15	Arachidonate 15-Lipoxygenase
ASICs	acid sensing ion channels
AST	anterior spinothalamic tract
ASTT	archispinothalamic tract
AT	Achilles tendinopathy
BCN	gene encoding biglycan
C	cytosine
Ca^{2+}	calcium ions
CACNA2D3	gene encoding the α2δ3 subunit of the voltage dependent calcium-channel complex
CACNG2	gene encoding the gamma-2 transmembrane AMPA receptor protein stargazin
CASP	caspases
CASP8	gene encoding caspase 8
Ca_v	voltage gated calcium channels
CD	colour Doppler
CD206	mannose receptor Cluster of Differentiation 206
CEUS	contrast enhanced ultrasound
CGRP	calcitonin gene related peptide

Cl^-	chloride ions
CM	centromedian
CNS	central nervous system
COL11A1	gene encoding α1(XI) collagen
COL11A2	gene encoding α2(XI) collagen
COL27A1	gene encoding α1(XXVII) collagen
COMT	catechol-O-methyltransferase
COMT	gene encoding catechol-O-methyltransferase
CON	control
COX-2	gene encoding cyclooxygenase-2
COX-2	cyclooxygenase-2
CPM	conditioned pain modulation
CTS	carpal tunnel syndrome
CXCL8	gene encoding interleukin 8
DIS-SRD	diffuse noxious inhibitory system-sub-nucleus reticularis dorsalis
DNA	deoxyribonucleic acid
DRG	dorsal root ganglion
ECD	Erdheim-Chester disease
ECM	extracellular matrix
ECRB	extensor carpi radialis brevis
ESWT	extracorporeal shockwave therapy
FBN2	gene encoding fibrillin-2
FM	fibromyalgia
fMRI	functional Magnetic Resonance Imaging

FOXO1	Forkhead box protein O1
G	guanine
GABA	gamma-Aminobutyric acid
GAGs	glycosaminoglycans
GDF	growth differentiation factors
HIF	hypoxia inducible factor
HMGB1	High mobility group box 1 protein
HPA	hypothalamic pituitary axis
IASP	International Association for the Study of Pain
IBS	irritable bowel syndrome
IFM	interfascicular matrix
IL-1	interleukin 1
IL10	gene encoding interleukin 10
IL13	gene encoding interleukin 13
IL-17A	interleukin 17A
IL1A	gene encoding interleukin 1 alpha
IL1B	gene encoding interleukin 1 beta
IL1R1	gene encoding interleukin 1 receptor type 1
IL1R2	gene encoding interleukin 1 receptor type 2
IL1RN	gene encoding interleukin 1 receptor antagonist
IL-1α	interleukin 1 alpha
IL-1β	interleukin 1 beta
IL-21/IL-21R	interleukin 21/interleukin 21 receptor
IL-33	interleukin 33

IL6	gene encoding interleukin 6
IL-6	interleukin 6
IL6R	gene encoding interleukin 6 receptor complex.
IL-8	interleukin 8
IL-lβ	interleukin 1 beta
IP_3	Inositol triphosphate
IQR	interquartile ranges
K^+	potassium ions
KCNS1	gene encoding potassium voltage-gated channel delayed rectifier subfamily S, member 1
K_v	voltage gated potassium channels
LBP	lower back pain
LET	lateral epicondylalgia
LST	lateral spinothalamic tract
M_2	muscarinic 2
MAF	minor allele frequencies
MAO	monoamine oxidase
MAPKs	mitogen-activated protein kinases
MRF	mesencephalic reticular formation
Mg^{2+}	magnesium ions
mGlut2	more metabotropic glutamate receptor 2
miRNA	micro ribonucleic acid
MMP1	gene encoding matrix metallopeptidase 1
MMP3	gene encoding matrix metallopeptidase 3

MMP8	gene encoding matrix metallopeptidase 8
MRI	magnetic resonance imaging
MRN	magnus raphe nucleus
mRNA	messenger ribonucleic acid
MSK	musculoskeletal
Na^+	sodium ions
$Na_v1.7$	voltage-gated sodium channels 1.7
NF-κβ,	nuclear factor kappa beta
NK cells	natural killer cells
NK-1R	neurokinin 1 receptor also known as tachykinin receptor 1 (TACR1)
NMDAR-1	N-methyl-D-aspartate-receptor subunit-1
NSAIDs	non-steroidal anti-inflammatory drugs
NSTT	neospinothalamic tract
OA	osteoarthritis
PAG	periaqueductal grey
PD	Parkinson's disease
PET	Positron Emission Topography
PF	parafasciculus
PG	prostaglandin
PGE2	prostaglandin E2
PGIS	prostacyclin synthase
PMNs	polymphonucleocytes
PNS	peripheral nervous system
PPI	present pain intensity

PPT	pressure pain thresholds
PRP	platelet rich plasma
PSFR	peak spatial frequency radius
PSTT	paleospinothalamic tract
PT	patellar tendinopathy
PTGS2	gene encoding cyclooxygenase-2 (also known as *COX-2*)
TNF-α	gene encoding tumour necrosis factor α
RA	rheumatoid arthritis
RC	rotator cuff
SC	spinal cord
SCN9A	gene encoding sodium voltage-gated channel alpha subunit 9
sf-BPI	short form Brief Pain Inventory
sf-MPQ	short form McGill Pain Questionnaire
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SNPs	single nucleotide polymorphisms
SNS	sympathetic nervous system
SP	Substance P
STT	spinothalamic tract
T	thymine
T2DM	type 2 diabetes mellitus
TAC-1	gene encoding substance P
TACR-1	gene encoding the substance P receptor 1
TEN	tendinopathy participants
TENS	transcutaneous electrical nerve stimulation

TGF-β	transforming growth factor beta
TIMP2	gene encoding issue inhibitor of metalloproteinases 2
TMD	temporomandibular disorder
TMJ	temporomandibular joint
TNC	gene encoding Tenascin C
TNF-α	tumour necrosis factor alpha
TRPV1	transient receptor potential cation channel subfamily V member 1
US	ultrasound
UTC	ultrasound tissue characterisation
VAMP	vesicle membrane protein
VAS	visual analogue scale
VDL	ventroposterolateral
VEGF	vascular endothelial growth factor
vGlut2	vesicular glutamate transporter 2
VISA- P	Victorian Institute of Sports Assessment – Patellar questionnaire
VISA-A	Victorian Institute of Sports Assessment – Achilles questionnaire
VPI	ventroposteroinferior
VPM	ventroposteromedial
WDR	wide dynamic range

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ETHICAL APPROVAL AND FUNDING

Ethical clearance and annual review to conduct the studies in this body of work were obtained from the Faculty of Health Sciences' Human Research Ethics Committee at the University of Cape Town (HREC 279/2016) (Appendix A).

This research was supported by funding from the National Research Foundation of South Africa (CPRR14072680498); the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant arrangement No 690850 (RUBICON) and the Margaret McNammara Education Grant.

THESIS ABSTRACT

Background

Achilles tendinopathy (AT) is a common injury in athletes and sedentary individuals, which presents as pain and loss of function in the lower limb. Tendon pathology can exist without pain, but the hallmark of the condition is pain, which is classically of insidious onset, related to loading activity and often resistant to treatment. While the biology of pain in general is well described, the mechanisms of pain in AT are not fully understood. Most commonly, the nociceptive driver associated with AT is thought to be a result of the structural changes that occur in the tendon or the inflammatory cascades that occur in the pathological tendon and/or reflective of altered central pain mechanisms. Evidence from other chronic pain conditions also shows that genetic variation explains, at least in part, some of the heterogeneity observed in chronic pain conditions. The presentation of chronic Achilles tendon pain is variable and therefore it is reasonable to propose that this variability may be influenced by a genetic component. The absence of a definitive cause or mechanism of pain in AT is reflected in the plethora of treatment strategies available to manage it, most of which are not universally effective. In order to improve the management of pain in chronic AT, it is imperative that its mechanisms be better understood.

Aims of the thesis

The aims of this thesis were therefore to characterise Achilles tendon pain using other pain questionnaires, to investigate the relationship between structural changes and central pain mechanisms with self-reported tendon pain. Additionally, the thesis sought

to evaluate the relationship between selected gene variants and pain in a cohort of recreational athletes with chronic Achilles tendinopathy using a candidate gene approach. Candidate genes: *COMT* rs4818 (C/G), *COMT* rs4633 (C/T), *TAC1* rs2072100 (C/T), *TACR1* rs3771829 (C/G) *and SCN9A* rs6746030 (G/A) were selected based on the biological function of their encoded proteins within the pain pathways. The objectives of the specific chapters which addressed these aims were:

- Describe Achilles tendon pain using multidimensional pain scales; the short forms of the McGill pain questionnaire (sf-MPQ) and Brief Pain Inventory (sf-BPI), as well as the Victorian Institute of Sports Assessment – Achilles questionnaire (VISA-A) **(Chapter 2)**.
- Evaluate the relationship between self- reported tendon pain, the grey scale ultrasound and colour Doppler characteristics in chronic AT **(Chapter 3)**.
- Evaluate the relationship between conditioned pain modulation and chronic AT **(Chapter 4)**.
- Explore and evaluate if variants in genes [*COMT* rs4818 (C/G), *COMT* rs4633 (C/T), *TAC1* rs2072100 (C/T), *TACR1* rs3771829 (C/G) *and SCN9A* rs6746030 (G/A)] involved in the pain pathways are associated with either self-reported tendon pain and/or conditioned pain modulation **(Chapter 5)**.

Methods

Two hundred and eighty-two (282) recreational athletes with at least one year's experience in their main sport were recruited for the studies in this thesis but fifty-two (52) were excluded for not meeting the inclusion criteria of the studies. Hence, 103

recreational athletes without a history of chronic AT (CON) and 127 participants clinically diagnosed with chronic AT (TEN) were included in the study. All participants completed demographic questionnaires on their medical, sporting, training, and injury history. Participants with AT (TEN) also completed the self-administered eight question VISA-A questionnaire, the sf-MPQ and the sf-BPI. Additionally, all participants had grey scale ultrasound (US) and colour Doppler (CD) assessments of both their tendons performed and had conditioned pain modulation (CPM) assessed using pressure and cold pain. Lastly, participants were genotyped for variants in *COMT* rs4818 (C/G), *COMT* rs4633 (C/T), *TAC1* rs2072100 (C/T), *TACR1* rs3771829 (C/G) and *SCN9A* rs6746030 (G/A) using standard PCR methods.

Data were analysed using Statistica Version 13.2.50. Normality of data was assessed using the Shapiro-Wilks test. Evaluations of differences between normally distributed quantitative data were conducted with the independent students t-test or one-way ANOVA, while Mann-Whitney-U and Kruskall-Wallis tests were used for non-normally distributed data. The Fisher's exact and χ^2 tests were used for categorical data. For post-hoc analyses, the Kruskal-Wallis associated multiple comparisons test with Bonferroni adjustment was used for quantitative data. For the genotyping data, Hardy–Weinberg equilibrium (HWE) was calculated using 'HardyWeinberg' version 1.6.3. package. The overall level of significance was set at $p<0.05$.

Results

From the sf-MPQ, the most frequent descriptors in the sensory index were 'aching' (n=73, 60.3%), 'tender' (n=64, 52.9%) and 'throbbing' (n=41, 33.9%). Words from the affective index of the sf-MPQ were seldom used to describe tendon pain. From the sf-

BPI, in addition to interfering with walking ability, AT pain also interfered with mood (n=61, 50.8%), sleep (n=40, 34.8%) and relationships with others (n=30, 25.0%). Additionally, there were weak to moderate correlations between corresponding indices on the sf-MPQ, VISA-A and sf-BPI (r^2>0.3; p<0.05) and the VISA-A scores were lower when participants reported severe tenderness (p=0.005), sharp (p=0.030) and/or stabbing pain (p=0.048) on the sf-MPQ.

On US and CD, the TEN participants had thicker tendons [median (IQR)] [TEN 6.3mm (5.4 - 7.9) vs CON 5.5mm (4.8 - 6.0), p<0,001]; relative abnormal ultrasound appearances (TEN 48.7%, n=38 vs CON 22.4%, n=19, p=0.001) and had more neovessels (TEN 20.5%, n=16 vs CON 1.0%, n=1, p=0.001) (p<0.001) than the CON participants. In the TEN group, no significant differences were noted between the self-reported total pain scores of the sf-MPQ, sf-BPI and VISA-A scales or their separate indices and tendon diameter, US abnormalities or presence of neovessels (p>0.05). However, the median interference index scores of the VISA-A questionnaire of participants with US abnormalities [median (IQR)] [35.5 (30.0 - 41.0), n=36] was significantly higher than those without US abnormalities [32.5 (26.0 - 37.0), n=39, p=0.046]. Additionally, participants from the TEN group who reported no stabbing pain, those who reported mild, moderate or severe stabbing pain on the sf-MPQ had significantly thicker tendons [median (IQR)] [6.0mm (5.2 - 7.6) vs 7.0mm (5.9 - 8.9), 7.7mm (6.2 - 9.1) and 6.3mm (4.9 - 7.4), p=0.037].

From the CPM analysis, participants with tendinopathy had a lower pressure pain threshold (PPT) before [median (IQR)] [TEN: 417kPa (364 - 516) vs CON 601kPa (459 - 724), p<0.001] and during [TEN: 458kPa (358 - 550) vs CON 633kPa (506 - 753),

p<0.001] the cold pressor test. However, there was no difference in the CPM effect between the two groups [median (IQR)] [TEN: 34kPa (-2 - 79) vs CON: 45kPa (4 - 94), p=0.490]. From the sf-BPI, PPT before the cold pressor test were significantly lower in individuals who reported mild to severe interferences in mood (p=0.023), general activity (p=0.038) and walking ability (p=0.004) when compared to those who reported no interferences. Pressure pain thresholds before the cold pressor test were also significantly lower in those participants who reported mild to severe pain at the time of testing (p=0.024) or reported moderate to severe pain on average (p=0.014) on the sf-BPI. Additionally, from the sf-BPI, a low CPM effect was significantly associated with mild to severe interference with sleep (p=0.043).

The genotype analysis showed that the median total scores of self-reported tendon pain from the sf-MPQ were significantly different (p=0.019) among the three *COMT* rs4818 (G/C) genotype groups [median (IQR)] [CC: 9.1 (4.0 - 13.0) n=61; CG: 7.3 (4.0 - 0.0) n=50; GG: 4.0 (1.0 - 5.0) n=7], with the CC genotype having a significantly higher pain score (p=0.018) than the GG genotype. No other associations were observed between genotype distributions of *COMT* rs4633, *TAC1* rs2072100, *TACR1* rs3771829, *SCN9A* rs746030 and the median self-reported total tendon pain scores for the sf-MPQ, sf-BPI, VISA-A, or their subscales.

Conclusion

The novel findings of this thesis suggest that the language of chronic AT pain ought to be further investigated as it may help extend our knowledge of the underlying mechanisms in chronic AT pain. In addition, that AT pain interferes with more than

physical and sporting ability should be considered in the overall management of this condition in athletes. While no associations were observed between imaging findings and tendon pain, the relationship between imaging findings and physical limitations suggests that using pain as a primary outcome measure in rehabilitation may be insufficient and highlights the need to further study the relationship between tendon structure, imaging and pain. Furthermore, impaired CPM was associated with interferences with sleep which suggests that, though not quite clear, some central mechanisms are at play in chronic AT pain. This finding also reaffirms the need to consider factors other than physical function in AT management.

Another novel finding of this thesis was the association between *COMT* rs4818 (C/G) and chronic tendon pain. This finding suggests that the catecholaminergic pathway is involved in the chronic AT pain pathway. *COMT* variants are associated with maladaptive coping mechanisms which may be important to consider in managing chronic pain conditions such as AT. In future, larger studies are required in order to replicate these findings and large, prospective cohort studies are required to confirm the role of genetic variation in chronic AT pain. Overall, the mechanisms of pain in tendinopathy are complex and not yet well described, emphasising the further need for multi-sectorial research.

CHAPTER 1

1.1. INTRODUCTION AND SCOPE OF THE THESIS

Achilles tendinopathy (AT) is a common sports injury that is particularly prevalent in those with a large running component[1], and presents as swelling, impaired lower limb function, and pain of insidious onset. While AT is a common overuse injury in the physically active population, it has also been reported in sedentary individuals[2–5]. There is an increase in the incidence and prevalence of overuse AT in both the physically active and sedentary populations due to the general increase in participation in sport and other physical activities. Additionally, incidences are increasing in professional athletes due to increased duration and intensity of training regimens and decreased rest times[1,6].

Achilles tendinopathy can be asymptomatic[7] and in some, rare, instances, spontaneous Achilles tendon rupture may be the first indication of pathology[8]. Indeed, studies on clinically asymptomatic tendons have shown that histopathologic changes can occur in tendons despite the absence of symptoms[9]. Despite the mismatch between tendon symptoms and pathology, in most instances, tendon pain is the primary reason for seeking healthcare or the modification or cessation of activity[10], as well as a major outcome measure in the management of AT[11]. The pain (i) is of insidious onset, (ii) is not proportionate to tissue damage, (iii) is difficult to manage, and (iv) can persist even after the resolution of other functional outcome measures[12,13]

Achilles and other tendon pain are therefore an enigma to clinicians and scientists alike, since they are difficult to manage with athletes continuing to experience symptoms despite aggressive treatment[14]. Examples of clinical treatment modalities for AT include (i) rest, (ii) anti-inflammatory drugs, (iii) corticosteroid injections, (iv) electrotherapy, (v) analgesics, (vi) soft tissue mobilisations (vii) exercise and (viii) surgery[15,16,25,26,17–24]. This abundance of therapeutic modalities for the management of what is structurally a trivial affliction is likely because the source and/or mechanisms of AT pain are currently not fully understood[27,28]. As a result, various hypotheses have been postulated to explain the origin of AT pain and its dissociation with structural abnormalities.

Traditional scientific dogma would have it that pain in tendinopathy arises by either one of two pathways: classical inflammation or matrix degeneration[29,30]. Following histopathological studies that failed to observe evidence of inflammation i.e. the presence of polymphonucleocytes (PMNs) in chronic tendinopathy[31], the inflammatory hypothesis was considered invalid in favour of the degenerative and failed healing theories[32]. Recent research, with more sensitive techniques, has however shown that inflammation is a feature of tendinopathy at least during the early stages of the condition and may have delayed downstream effects that are degenerative[33,34]. Some authors attribute tendon pain to structural abnormalities as observed with imaging modalities[35] or to the nerve ingrowths that accompany the neovessels that are a feature of chronic tendinopathy[36]. Others still have proposed that neuropeptides and other tendon metabolites such as Substance P (SP) and glutamate may be responsible for the pain in AT[37,38]. While others postulate that changes in central pain modulation are responsible for chronic tendon pain[39,40] These various hypotheses

demonstrate that in spite of decades of research, the mechanisms of tendon pain are still not clearly described.

Although the aetiology of AT is poorly understood, it is well established, as summarised in a number of review articles, that common DNA sequence variants modulate risk of injury[41–43]. Previous research has focused on variants within genes that encode for structural components of the tendon extracellular matrix (ECM) or regulate biological processes within tendons[42]. Since tendon pain shares some characteristics with other types of chronic musculoskeletal (MSK) pain[44], genetic variants, which have previously been associated with pain perception, modulation, transduction, transmission and conduction in other conditions[45–47], could modulate the inter-individual variability in the pain experiences during chronic AT. These associations may, at least in part, contribute to the poor correlation between tendon pathology, pain and disability[13]. To the author's knowledge, the association of genetic variants with pain has not been previously investigated in chronic painful AT. However, this has been extensively investigated in other conditions, which has contributed to elucidating the mechanisms of pain and hence inform treatment options[48].

The objective of this body of work was therefore to review the current knowledge and propose potential research targets on mechanisms of pain in chronic AT and investigate three selected hypotheses in a cohort of recreational endurance runners; namely, tendon structural changes, changes in central pain mechanisms and genetic variation as potential sources of tendon pain. Firstly, **Chapter 1** summarised the current knowledge on pain mechanisms in tendinopathy. This includes a review of normal tendon structure and function, focusing on the Achilles tendon *(Section 1.2.)*,

as well as the clinical features of tendon pain *(Section 1.3.).* The biology of pain (*Section 1.4.)* and the current hypotheses of potential contributors to Achilles tendon pain are evaluated *(Section 1.5.).* Finally, the review drew upon previous research in pain genetics to provide a theoretical framework for genetic variation as a possible contributor to chronic Achilles tendon pain and propose possible biological mechanisms based on these *(Section 1.5.4.).* Following the literature review, the thesis provides a novel characterisation of Achilles tendon pain using multiple pain scales in **Chapter 2** followed by an examination of three of the hypotheses with relation to pain in AT in **Chapters 3** to **5**, and a final integrated discussion **(Chapter 6).** The structural and neovascular hypothesis is examined in **Chapter 3**, the central pain hypothesis in **Chapter 4** and finally, genetic variation and susceptibility to pain in **Chapter 5**.

1.2. ANATOMY OF THE ACHILLES TENDON

1.2.1. Gross Anatomy of the Achilles Tendon

The Achilles tendon (also known as the calcaneal tendon) is named after Achilles, son of Thetis and a Greek hero of the Trojan war whose only vulnerable body part was his heel that his mother held him by when she dipped him in the river Styx to immortalise him[49]. Though strong, it is the most frequently injured tendon in the body[50]. It originates in the middle of the calf as a confluence of the tendinous aponeuroses of the gastrocnemius and soleus muscles in the posterior superficial compartment of the leg[49,51] (Figure 1.1). As the tendon courses from its proximal attachment to its distal attachment, its fibres rotate up to 90° making the medial side more superficial (posterior) and the lateral side more deep (anterior)[52]. It then inserts into the posterior surface of the calcaneus, distal to the postero-superior calcaneal tuberosity[53,54]. The fibres from the gastrocnemius insert into the posterolateral aspect while those from the soleus insert posteromedially. The enthesis is formed by the osteotendinous junction of the Achilles tendon, sesamoid fibrocartilage, periosteal fibrocartilage and the tip of Kager's fat pad[53,55,56]. The relative contribution to the tendon by the two muscles is variable. In most instances (52%), the soleus muscle is the major contributor, in 35% of cadaveric specimens the contribution is equal and rarely (13%) is the gastrocnemius the major contributor[49,52,57].

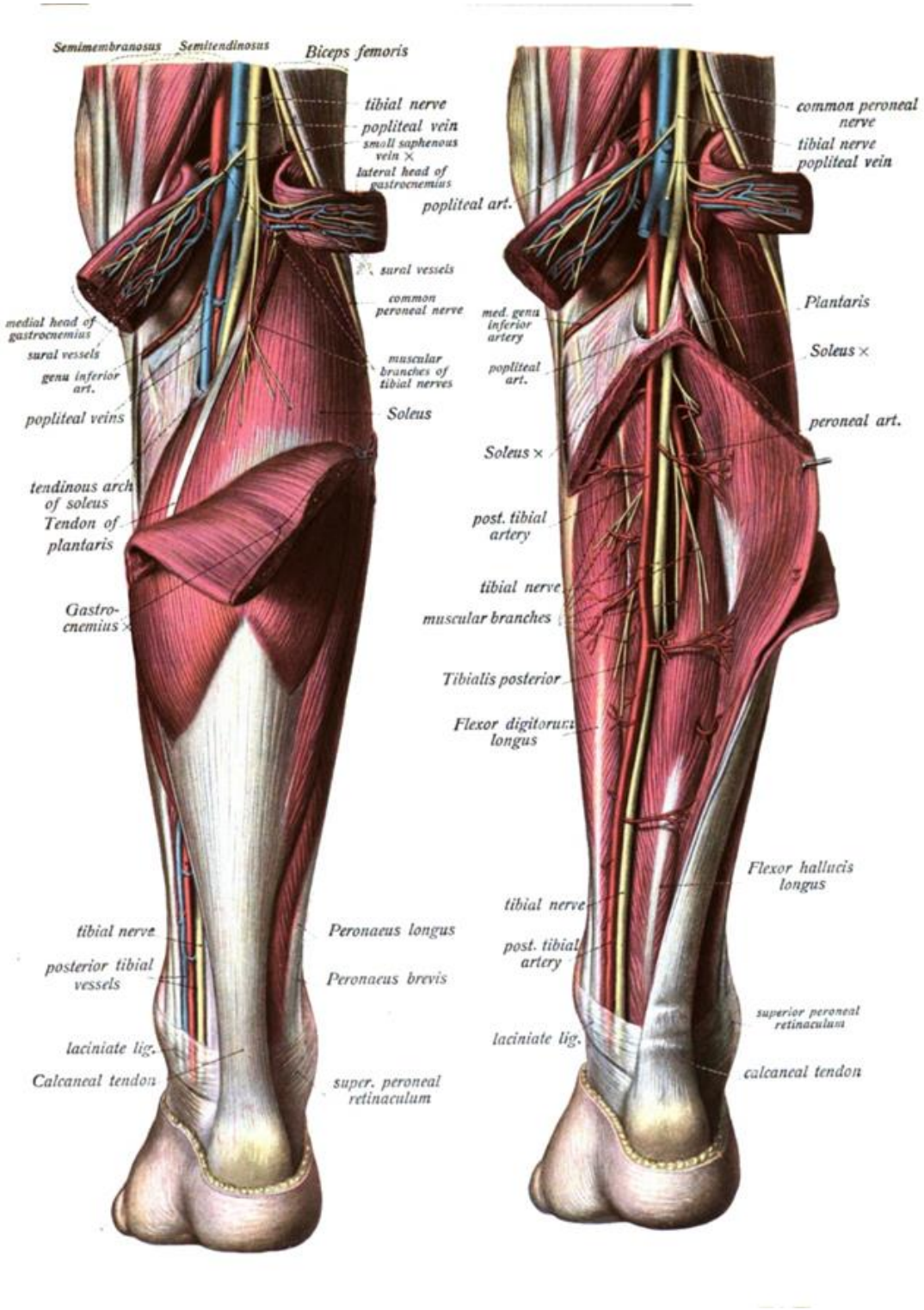

Figure 1.1: The Achilles tendon and its anatomical relations ((2020, March 20). *Wikimedia Commons, the free media repository*. Retrieved 08:18, August 3, 2020 from [commons.wikimedia.org])

It is related laterally to the sural nerve about 10cm above its calcaneal insertion. Distally, it has the retrocalcaneal and adventitious bursae located anteriorly and posteriorly to it, respectively[58]. Also anterior to the tendon is the muscle belly of flexor hallucis longus while the neurovascular bundle lies medial to it and the plantaris muscle, which sometimes, but not always, inserts on the medial aspect of the calcaneum[57,59,60]. The Achilles tendon is arbitrarily divided into the proximal, mid- and distal sections. On average, the tendon is approximately 15cm long (mean 12-26cm) and 6.8cm wide[49,61]. The gastrocnemius component of the tendon is often longer than the soleus component; 11-26cm and 3-11cm, respectively[54]. On cross section, the musculotendinous origin is rectangular and thick; the midsection is oval and narrow while the tendo-calcaneal insertion is flared and flattened about 4cm above its insertion[53,54]. The Achilles tendon does not have a synovial sheath; however, it is covered by the peritenon, which is a single cell layer that enables smooth gliding during activity and provides vascular support to the tendon[51,62] as well as promotes healing[63,64]

The blood supply to the Achilles tendon declines with age[57], is derived from the intrinsic systems at the musculotendinous and osteotendinous junctions or through the extrinsic system via the paratenon[61]. The intrinsic system mainly supplies the proximal and distal components of the tendon and is predominantly from the recurrent branch of the posterior tibial artery, which supplies the majority of the tendon and the peroneal artery, which, through anastomoses with the posterior tibial artery, perfuses the lateral aspect of the tendon[49,54,61,65](Figure 1.1).The former also supplies the peritendinous tissue. Additionally, blood supply to the proximal third of the tendon derives from vessels supplying the muscle bellies continuing into the endotenon and that to the

distal third derives from the *rete arteriosum calcanere,* which are small feeder arteries from the fibular and posterior tibial arteries[54]. The watershed area (midsection) of the tendon is also supplied by the peroneal artery and is the site of greatest torsion in the tendon[49,65,66].

The nerve supply to the Achilles tendon is from branches of the nerves of the attaching muscles and the cutaneous nerves, in particular the sural nerve[49,50]. The nerves mainly terminate on the surface of the tendon or paratenon but can coalesce to form a longitudinal plexus that follows the vascular channels and largely supplies afferent fibres in the tendon[67]. Tendons have both sympathetic and parasympathetic nerve fibres and all the four types of receptors i.e. Ruffini and Vater-Pacinian corpuscles, myelinated Golgi tendon mechanoreceptors and unmyelinated nociceptors[52].

1.2.2. Micro-anatomy of the Achilles Tendon

A healthy Achilles tendon is brilliant white in colour and is fibroelastic in nature. The major cell type in the Achilles tendon are the tenoblasts and tenocytes, which account for 90- 95% of the tendon cellular content. Chondrocytes, vascular cells, synovial cells and smooth muscle cells constitute the remainder[49,61,68]. In normal tendon, all the cellular components are arranged in an organised pattern. The tenocytes synthesise all the components of the extracellular matrix of which collagen (65-80%) and elastin (1-2%) are the major macromolecular components. The extracellular matrix also contains approximately 68% water[49,68]

Type I collagen accounts for up to 90% of the dry mass of the tendon, with the remaining 10% consisting of trace amount of functionally important types III, V, IX, X, XI, XII collagen and other non-collagen molecules[69–71]. In particular, collagen type III production is increased in pathological tendons or during healing and, compared to type I collagen, is less resistant to tensile force[49]. The enthesis of the Achilles tendon, which in addition to normal tensile forces to the midportion of the Achilles, also has to resist compressive forces, has a fibrocartilage structure and contains types II, XI and X collagen as well as other non-collagen molecules found in cartilage[61,69,72].

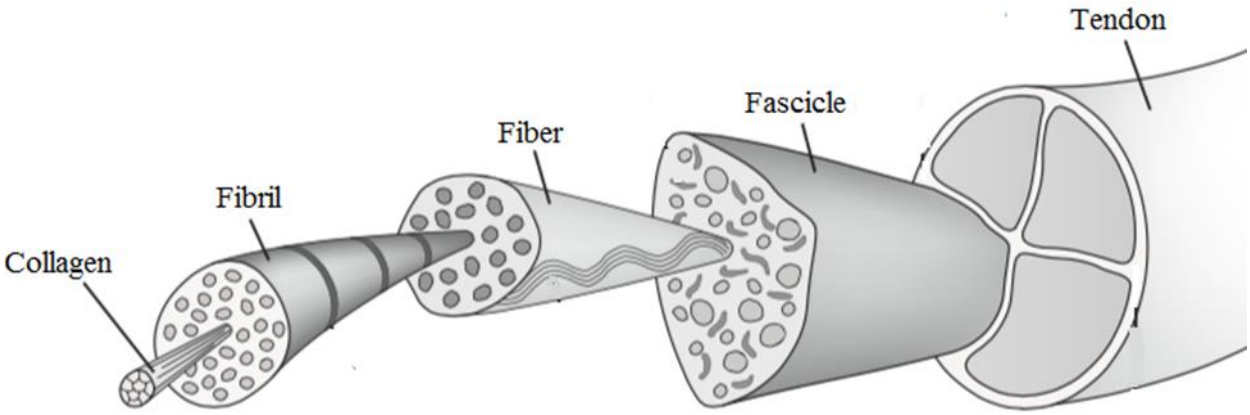

Figure 1.2: Hierarchical structure of the tendon, which contributes to the tendon's mechanical competency ((2020, March 20). *Wikimedia Commons, the free media repository*. Retrieved 08:18, August 3, 2020 from [commons.wikimedia.org])

Within the tendon, type I collagen, together with the other fibrillar forming and fibril associated collagens are sequentially arranged in hierarchical levels of increasing complexity and strength that consist of fibrils, fibres, fascicles and ultimately, the macroscopic tendon (Figure 1.2).This hierarchy confers strength to the tendon by aligning all structural levels parallel to the line of greatest tension and ensures that

failure of one component will not lead to macroscopic failure[58,61]. The collagen fibrils together with fibroblasts are tightly packed in parallel bundles containing nerve, blood and lymphatic vessels forming fascicles. These are enclosed in endotenon, which acts as a conduit for blood vessels, lymphatics and nerves and aggregate to form the macroscopic tendon[73] (Figure 1.3).

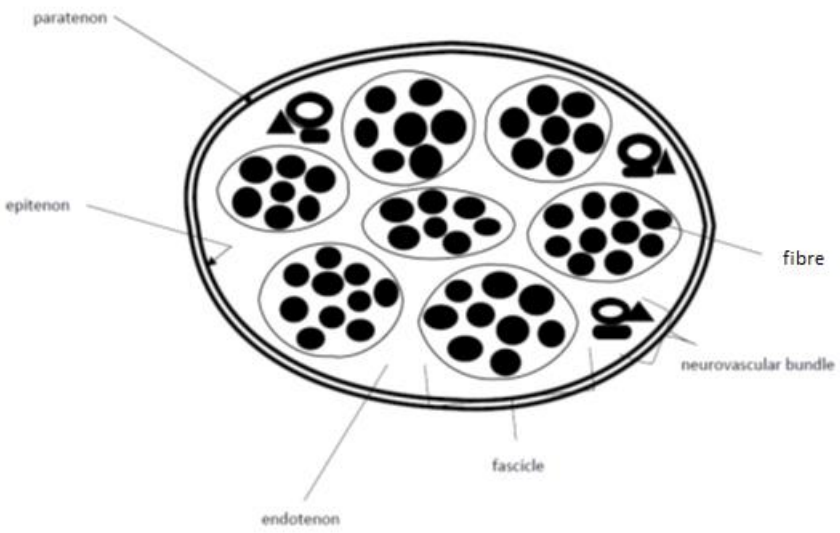

Figure 1.3: A schematic diagram of the cross section of a tendon showing the loose paratenon under which is the epitenon. The endotenon contains blood vessels, lymphatic vessels and nerve fibres. The collagen fibrils are tightly packed in the fascicle.

The tendon is in turn wrapped in epitenon, a fine, loose areolar connective tissue. Unlike other tendons, the Achilles tendon lacks a true synovial sheath. Instead, superficial to the epitenon is the paratenon which is also a layer of loose, areolar connective tissue consisting of types I and III collagen, elastic fibrils and synovial cells[74,75]. The epitenon and the paratenon sandwich a thin fluid layer to decrease friction during tendon motion[49]. Between the tendon fascicles is the highly cellular interfascicular matrix (IFM) or the endotenon (Figure 1.3). It is a loose connective tissue composed of collagen type III, proteoglycans, and is synthesised and

maintained by interfascicular fibroblasts[76]. In the Achilles tendon, it is proteoglycan and elastin rich, which is ideal for its main purpose: facilitating sliding and recoil of the fascicles, which enables high levels of extensibility necessary for Achilles tendon function[78,80]. It is less dense than fascicular tissue and is continuous with the peritendon structures, which ensures mechanical integrity of the whole tendon structure despite primary discontinuities in the tendon structure[82,83]. With age, and in pathology, the IFM becomes stiffer and less fatigue resistant, potentially explaining why older tendons become more injury prone[86,88,90].

The collagen fibrils, elastin, tendon cells and other non-fibre forming proteins are embedded in a gel-like medium known as the ground substance. The ground substance, which enables diffusion of water-soluble molecules, assists with cell migration, amongst other functions, consists predominately of water, with its major macromolecular constituents consisting of proteoglycans (PGs) and glycosaminoglycans (GAGs)[77]. PGs are a structurally and functionally diverse family of molecules consisting of a core protein with one or more covalently attached GAG chain(s), which constitute between 0.2- 0.5% of the tendon's dry weight. The negatively charged GAG chains make the proteoglycans strongly hydrophilic, which binds to water. The bound water to the high GAG content in aggrecan enables tissue to resist compressive forces while decorin permits fibrillar slippage by mediating lateral fibril growth to ensure the regular arrangement of collagen fibrils[79,81]. These anatomical features are often altered in tendinopathy and may, as described in subsequent sections of this review, explain some of the clinical features of the condition such as pain.

Finally, since the cellular content of tendons is low compared to other tissues, the basal metabolic rate of the Achilles tendon is low. The low metabolic rate allows the tendon to maintain postural tension for extended periods of time without risking ischaemia. It, however, also slows down healing in the event of injury or insult, as well as adaptation to load and other stimuli as shown in carbon-14 bomb pulse dating studies[84]. The precise mechanisms of how or why the macro- and microanatomy of the tendon changes in pathology is still unclear and a subject of ongoing research. Even more unclear is how the changes in tendon structure relate to common clinical symptoms of tendinopathy such as pain.

1.3. CLINICAL FEATURES OF ACHILLES TENDON PAIN

Pain is a hallmark of AT and is currently the most accurate clinical indication for the condition[85]. It is the cardinal symptom for which patients seek help and is the most common measure of disease severity[70]. Pain in AT typically presents as pain localised to a point that is 2-6cm proximal to the calcaneal insertion and is often felt as morning pain and at the commencement of exercise[87]. The pain is of insidious onset and long duration with no definitive triggering event or trauma[89] except increases in the magnitude or rate of load application and hence it can be associated with an increase in training intensity or duration, as well as change in training technique[91].

Achilles tendon pain may also be associated with focal point tenderness to palpation [92–95]. On palpation, this area's tenderness significantly decreases or disappears when the tendon is put under tension[96]. Sometimes, though rarely, it radiates to the neighbouring structures but usually the exact location of the pain adds diagnostic

accuracy to clinical tests[39,97]. Achilles tendon pain may also result in impaired lower limb function such as gait abnormalities from reduced gastro-soleus endurance in long standing tendinopathy[91,95,98]. Though not widely studied, Achilles tendon pain also has significant psychological burden[99,100]. The main assessment tool for the severity of pain in AT and its effect on activity is the VISA-A[11]. However, the VISA-A scale has several limitations as it is rather unidimensional in its interrogation of tendon pain. These limitations will be discussed in further detail in **Chapter 2**.

Achilles tendon pain is seldom experienced at rest, instead it is associated with rapid activity that involves the repetitive energy storage and release that occurs during running. It appears instantly with loading and disappears almost immediately with unloading[101]. Tendon pain can also be felt after prolonged inactivity such as extended sitting or early in the morning[102]. It can be reproduced with resisted muscle movement and active stretching of the tendon[102]. Characteristically, the pain improves with continued loading exercise ('warm-up' phenomenon)[87]. In fact, during the initial stages of the condition, the pain or discomfort is experienced at the beginning of exercise and disappears as it warms up. As the condition progresses, however, pain is felt during exercise and can result in the cessation of activity[89,95] or even premature retirement from professional sport[103]. Another characteristic of tendon pain is that it is famously resistant to most analgesic therapies that are currently available on the market[104,105].

Histological examination of chronic painful tendons shows collagen disorientation and fibre separation, increased proteoglycan content, areas of hyper- and hypo-cellularity, as well as neovascularisation[106–110]. On physical examination and/or imaging, the painful tendon is often thicker in the coronal and sagittal planes. Additionally, other

observations include altered echogenicity[69,106,111] and neovascularisation on CD[112], which are associated with pain. However, these imaging findings have been observed to be unrelated to self-reported pain in some cohorts[12], did not provide any prognostic value in others[113] or were altogether inconclusive[114]. However, it is important to note that not all posterior leg pain that presents proximal to the calcaneal insertion is mid portion AT.

Posterior heel and ankle pain are frequent presentations in primary care. Therefore, causes of this pain other than AT should be considered. In particular, the anatomical relations of the Achilles tendon such as the paratenon, gastrocnemius and soleus muscles, plantaris tendon, the sural neve, Kager's fat pad and the bursae can be a source of posterior heel pain which may be misdiagnosed as AT[27,115,116]. Additionally, partial rupture of the tendon can be mislabelled as AT. However, for these differential diagnoses, the patient history, results of clinical diagnostic tests and other associated symptoms such as pain worsening with activity, pain accompanied by burning/tingling or imaging observations would rule out AT[117]. Posterior heel pain can also be a result of vascular compromise (e.g deep vein thrombosis), infection (e.g. cellulitis); neoplasias, iatrogenic injury; congenital abnormalities; autoimmune conditions (e.g. Erdheim-Chester disease (ECD) or endocrine/ metabolic disorders (tendinous xanthoma)[117–122].

It is evident that tendon pain is a major problem in clinical practice and therefore a thorough understanding of its mechanisms would be invaluable to clinicians and researchers in developing effective treatment methods. Hence, an understanding of

general pain concepts will provide a context from which to evaluate and study tendon pain and will therefore be reviewed in the next section.

1.4. THE BIOLOGY OF PAIN

Pain is defined by the International Association for the Study of Pain (IASP) as '*an unpleasant sensory and emotional experience associated with actual or potential damage or described in terms of such damage. Pain is always subjective, and each individual learns the application of the word through experiences related to injury in early life.*'[123,124]. This distinguishes it from nociception, which is the neural process involving the transduction and transmission of a noxious stimulus to the brain. Pain is a result of a complex interaction of signalling systems, modulation from higher centres and the individual's unique perception[124]. It is often an indication of physical damage. However, the pain experience does not necessarily mean that damage has occurred, and the perceived pain may be far greater or less than the actual extent of the injury or disease would suggest. Pain is, in addition to being a physical experience, an inner experience generated within the brain by a combination of factors that include socio-cultural factors, which have an impact on the suffering experienced[125,126]. Pain can further be described as physiological or pathological. Physiological pain is directly elicited by application of a noxious stimulus to normal tissue, reflects impending or actual tissue damage and hence is useful and serves an evolutionary purpose. Pathological pain exists in the absence of a known or direct noxious stimulus and is associated with functional alterations in the properties of the nervous system[127–129]. This can occur as a consequence of damage to nerve fibres leading to increased spontaneous firing or alterations in their conduction or neurotransmitter properties[130].

Additionally, repetitive C fibre activation by noxious stimuli leads to a reduction in local inhibition by the neurotransmitters gamma-Aminobutyric acid (GABA) and glycine as well strengthening of excitatory synaptic connections. The collective result is that the sensory threshold for pain signalling is lowered[125,126,131]. Structural rewiring in the dorsal horn of the spinal cord (SC) and cortical remapping can also occur in response to a persistent barrage of nociceptive signalling from the peripherals[124]. Tendon pain can be classified as pathological pain as changes in the peripheral[40,132] and central nervous systems[39,133] have been noted. Additionally, tendon pain can persist for years and the observed pathology is not proportionate to the pain[13]. However, tendon pain is generally confined to the tendon itself, and does not refer to its anatomical relations. It is also associated with mechanical loading[87,101], which would fit more with physiological pain. In order to explore the cause(s) of tendon pain further, an understanding of the biological concepts of pain is therefore helpful.

1.4.1. Pain Sensation

The nociceptive stimulus is sensed by specific receptors with free nerve endings called nociceptors. These are unspecialised, free and unmyelinated or poorly myelinated nerve endings that transduce a variety of stimuli into nerve impulses[134]. There are two main types of nerve fibres that carry nociceptors: small diameter unmyelinated C fibres and larger diameter thinly myelinated Aδ fibres[129,134]. The Aδ fibres, which release glutamate, are small diameter (1-5μm), myelinated nerve fibres which transmit nociceptive signals from the peripheral nerves to the spinal cord at velocities of between 6 and 30ms^{-1} [135,136]. These are responsible for the initial fast onset, sharp pain that is felt within 0.1s of exposure to the noxious stimulus particularly of a

mechanical or thermal nature. The C fibres, which release glutamate and SP, are even smaller diameter (0.4-1.2μm), unmyelinated nerve fibres, which transmit a longer lasting diffuse and slow nociception after a latency period of at least 1s at maximum speeds of $3ms^{-1}$[123,137]. Slow pain is often associated with tissue damage and while it can be elicited by persistent mechanical or thermal stimuli, it is often a result of chemical stimuli[136]. Peripheral activation of nociceptors can be from various thermal, mechanical or chemical stimuli[138,139]. Additionally, nociception can be via the stimulation of low-threshold mechanoreceptors (LTMRs) such as acid sensing ion channels (ASICs), which have been demonstrated to serve as touch receptors as well as play an important role in acute pain signalling[140].

1.4.2. Pain Transmission

Nociceptive transmission begins when noxious stimuli cause a change in the cell membrane conformation of the nociceptors. This change in conformation leads to changes in the relative permeability of the neuronal membranes of the nociceptors to various ions such as K^+ and Na^+ and leads to the formation of a nociception related action potential in the Aδ or C fibre nerves through the flow of ions through voltage gated sodium or potassium channels or acid sensing ion channels[130]. This action potential travels down the Aδ or C fibres as a wave of alternate depolarisation and repolarisation of the membrane through the opening of Na^+ and K^+ ion channels that are ubiquitous in the membrane[135]. This continues to happen until the signal reaches a junction to the next neuron in the chain called the synapse. All the terminations and connections that occur from the first, second, third order neurons and ultimately the cerebral cortex occur at junctions known as synapses[139] (Figure 1.4).

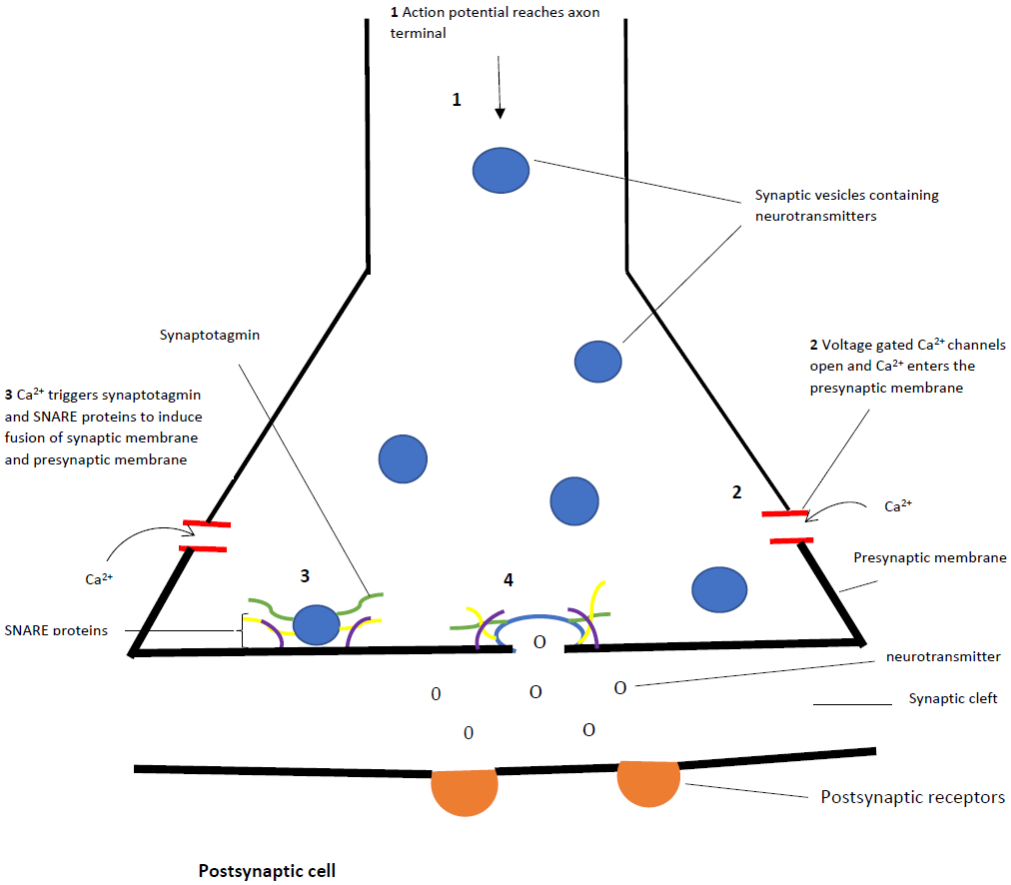

Figure 1.4: A schematic example of a chemical synapse. The action potential from the peripheral stimulus reaches the presynaptic terminal (1) where it causes the opening of the voltage gated Ca^{2+} channels (2). The Ca^{2+} influx ultimately leads to the activation of SNARE proteins in the presynaptic membrane which allows fusion of the presynaptic and vesicle membranes (3) which results in the exocytotic release of the neurotransmitter into the synaptic cleft. (SNARE= Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor). *Adapted from Windmaier et al., 2013.*

Synapses can be either electrical or chemical. The latter group form the majority of synapses. The presynaptic membrane contains large numbers of voltage gated Ca^{2+} channels[128]. When the wave of the action potential from the nociceptive stimulus reaches the synaptic bouton of either the Aδ or C fibre, it spreads over the synaptic terminal, depolarises the membrane and causes the Ca^{2+} channels to open and allow

a rapid influx of Ca^{2+} down its concentration gradient into the presynaptic terminal[126,136]. Once inside the presynaptic cell, Ca^{2+} activates calmodulin, which in turn activates calmodulin dependant kinase II. The latter then phosphorylates synapsin, a vesicle membrane protein (VAMP) and reduces its affinity for the synaptic vesicles, which are then released. The vesicles are chaperoned to the active zone of the presynaptic neuron by two G proteins, rab3A and rab3B, in addition to synapobrevin and synaptotagmin. The latter are Ca^{2+} sensitive v-SNARE proteins on the synaptic vesicles that recognise the t-SNARE protein, syntaxin, on the presynaptic membrane and complete the docking of the synaptic vesicle to the presynaptic membrane. Following docking of the vesicle, there is a second influx of Ca^{2+}, which causes fusion, the formation of a fusion pore by synaptophysin and physophilin and ultimately exocytosis of the vesicular contents into the synaptic cleft[139].

Synaptic vesicles can be one of three types. They may contain neurotransmitters (glutamate, glycine, acetylcholine and GABA), catecholamines (adrenaline, noradrenaline and dopamine) or neuropeptides (e.g. SP)[126]. Slow C fibre neurons secrete SP from their synaptic vesicles[135]. This diffuses across to the postsynaptic membrane's postsynaptic density and binds to G coupled receptors and proceeds to initiate Inositol triphosphate (IP_3) dependant cellular reactions. Catecholamine vesicles release adrenaline, noradrenaline or dopamine into the cleft, these utilise cyclic AMP (cAMP) as a second messenger. The catecholamines are then either methylated by catechol-O-methyltransferase (COMT) or oxidised by monoamine oxidase (MAO) into inactive metabolites or reabsorbed into the presynaptic membrane[139].

Once the neurotransmitter substance has bound to its respective receptor on the postsynaptic membrane, it facilitates changes in the Cl^-, Na^+ and K^+ permeability characteristics of the postsynaptic membrane. Excitatory neurotransmitters such as glutamate open cation channels such as voltage gated sodium and potassium channels, causing an influx and efflux of Na^+ and K^+, respectively. This depolarises the postsynaptic membrane and continues the orthodromic propagation of the action potential from the presynaptic neuron[136]. On the other hand, inhibitory neurotransmitters such as GABA open anion channels such as Cl^-. This hyperpolarises the postsynaptic membrane and decreases its excitability and hence the propagation of the action potential. This action potential is relayed in an all or nothing fashion up the afferent nociceptive neuron, which terminates in the dorsal horn of the spinal cord by synapsing with second order neurons. At this level, interactions occur between excitatory (glutamatergic) and inhibitory (GABAergic) interneurons and descending inhibitory tracts from higher centres exert their effect[124,141]. At the level of the spinal cord, the passage of nociceptive signals from the periphery is controlled by inhibitory control from the higher centres, activation of Aβ collaterals and segmental modulation by a variety of mechanisms such as endogenous opioid and cannabinoid systems[131].

The primary afferent nociceptive fibres enter the spinal cord from the peripheral tissues through the posterior root ganglion and divide into ascending and descending branches[123,142]. These branches then travel over one or two segments and form the posterolateral tract of Lissauer. These first order Aδ and C fibre neurons then synapse with second order neurons in laminae I and II (substantia gelatinosa)[135] in the posterior grey column though some Aδ fibres pass as far deep as lamina V while fibres of first

order neurons from the head, neck and oral cavity have cell bodies in the trigeminal nerve[136,137]. The latter decussate and ascend as the trigeminothalamic pathway. The neurotransmitter of choice here is SP.

The second order neurons then give rise to axons, some of which decussate immediately in the anterior grey and white commissure within the spinal segment of the cord and ascend to the central nervous system were they synapse with third order neurons as a bundle of homogenous fibres known as the spinothalamic tract[124,134]. The spinothalamic tract can be further divided into three pathways: a neospinothalamic tract (NSTT), paleospinothalamic tract (PSTT) or the archispinothalamic tract (ASTT)[142].

The neospinothalamic tract has very few synapses and is constitutively the classical lateral spinothalamic pathway. The lateral spinothalamic tract (LST) is considered as one of the most important pathways in nociception and ascends to the thalamus in the contralateral anterolateral quadrant of the spinal cord[123,139] (Figure 1.5). The first order neurons of the LST terminate in the Rexed layer I neurons (lamina marginalis) of the dorsal horns[143]. The LST is located anterolaterally in the spinal cord though some of the fibres run in the dorsal portion of the cord[139,142]. As the lateral spinothalamic tract ascends through the medulla oblongata, it lies in proximity to the lateral surface and between the inferior olivary nucleus of the spinal tract of the trigeminal nerve. It is here that it becomes part of the spinal lemniscus together with the spinotectal and anterior spinothalamic tract. The spinal lemniscus ascends through the posterior aspect of the pons and lies in the tegmentum lateral to the medial lemniscus in the midbrain. The majority of the fibres of the LST synapse with third order neurons in the ventral

posterolateral nucleus of the thalamus, which are the specific sensory relay nuclei in the thalamus[135,139]. Specifically, fibres from the lower limb and below the neck terminate in the ventroposterolateral (VPL) and ventroposteroinferior (VPI) nuclei of the thalamus[123,142]. The second order neurons of Aδ fibres terminate in the ventroposteromedial (VPM) thalamus and the C fibres terminate in the parafasciculus (PF) and centromedian thalamus (CM). They also synapse in the periaqueductal grey (PAG), reticular formation of the brainstem and the hypothalamus[135,142]. These third order neurons, particularly those terminating in VPM and VPL, now pass though the posterior limb of the internal capsule and corona and radiate to project to the somatosensory cortices SI and SII in the post central gyrus for conscious pain processing[142]. This pathway uses glutamate as a neurotransmitter and is responsible for discriminative properties of pain, i.e. for the immediate awareness of a nociceptive sensation and its exact location[134].

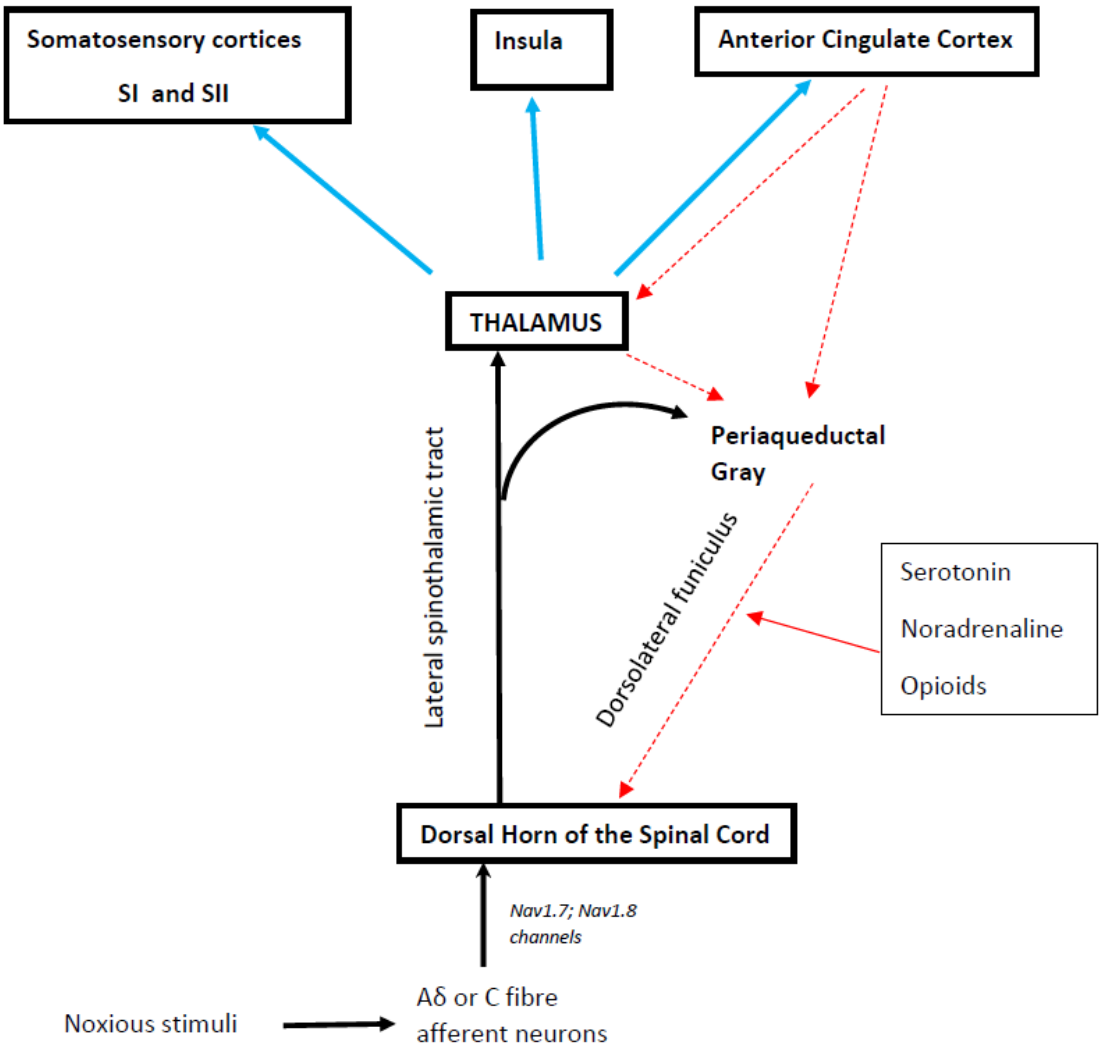

Figure 1.5: Schematic representation of the reception of the nociceptive stimulus from the environment and its transmission through the central nervous system (solid black lines). The main tract that carries nociceptive signals from the dorsal horn is the lateral spinothalamic tract which provides some input to the periaqueductal grey. Once the signal reaches the thalamus, it is projected to the higher cortices in the brain such as the somatosensory cortices, insula, and the anterior cingulate cortex where pain perception occurs (solid blue lines). Descending input from the higher cortices to the periaqueductal grey leads to suppression of pain signals (broken red lines) which results in analgesia. This process is dependent on catecholamines (noradrenaline), serotonin and opioids (β enkephalins, β endorphins).

The paleospinothalamic tract is phylogenetically old, hence the prefix. It transmits nociception mainly from the C fibres although it also transmits signals from Aδ fibres[136]. The majority of the first order neurons of this pathway make synaptic connections in Rexed layer II (substantia gelatinosa) and the second order neurons make synapses in laminae IV-VIII. Some fibres pass through to lamina V where the last neurons give rise to long axons that join the neospinothalamic tract[142]. The second order neurons also receive input from mechano- and thermoreceptors; some of the nerve cells in the PSTT are wide dynamic range nociceptors (WDR), which respond to various stimuli. Most of their axons cross and ascend in the spinal cord as the anterior spinothalamic tract (AST) where they primarily transmit tactile information. The PSTT terminates widely in the brain stem and only 25% of its fibres proceed to the thalamus[142]. Instead, most of its fibres make connections in the mesencephalic reticular formation (MRF) and the PAG as the spinoreticular tract; in the tectum as the spinotectal tract and in the PF-CM complex as the spinothalamic tract. Some of the PSTT fibres decussate at the level of the cell bodies but some ascend ipsilaterally and synapse in the somatosensory cortex SII. Positron Emission Topography (PET) and functional Magnetic Resonance Imaging (fMRI) studies have validated this by demonstrating that pain activates multiple areas in the cortex including the cortical areas SI and SII and the anterior cingulate gyrus on the contralateral side to the pain[123]. Additionally, the mediofrontal, supplementary motor and insular cortices as well as the cerebellum are also activated. The PSTT also activates the brain stem nuclei, which are the origin of the descending pain suppression pathways while the spinoreticular formation also connects extensively with the cingulated gyrus, limbic system and insular cortex, which process the motivational-affective components of

pain[144]. Nociceptive localisation by the PSTT is very poor due to the diffuse, multisynaptic nature of this pathway[145].

The ASTT pathway is a multisynaptic diffuse tract. The first order neurons make connections in the substantia gelatinosa and ascend to laminae IV and VII. From these laminae, fibres ascend and descend in the spinal cord via the propriospinal pathway surrounding the grey matter to synapse in the MRF-PAG area. Further diffuse pathways ascend to the intralaminar areas of the thalamus and send collaterals to the hypothalamus and limbic system nuclei[126]. The fibres of the ASTT mediate visceral, emotional, and autonomic reactions to pain. This mediation is part of a number of instances of how pain is modulated in the CNS[146].

It is in the higher cortices that the cognitive and affective processing of pain and the accompanying behaviour changes occur[144]. Sufficient activation of the nociceptive system may give rise to the perception of pain, an unpleasant sensory experience with both sensory-discriminative and affective components[127]. Also important in nociception and pain are the tracts involved in descending pain modulation where the catecholaminergic and serotonergic systems play a crucial role in this facilitation or inhibition of nociceptive transmission[147]. At the level of the midbrain and brainstem, descending inputs from the brainstem nuclei stimulate dorsal horn interneurons to release endogenous opiate analgesics to dampen the nociceptive signal as part of the internal analgesic system[126].

1.4.3. Central Pain Modulation

1.4.3.1. The Analgesia System

Laboratory experiments have shown that stimulation of certain areas of the brain can attenuate/abolish pain[148,149]. These areas are collectively called the analgesia system and consist of the PAG and periventricular area in the midbrain; the midline nuclei of the brainstem such as the magnus raphe nucleus (MRN), dorsal raphe nucleus and nucleus reticularis paragigantocellularis and the pain inhibitory complex in the dorsal horn of the spinal cord[127,142,144] (Figure 1.5). The PAG modulates nociceptive transmission by inhibiting primary afferent transmission in the dorsal horn. Its neurons project directly to and activate two groups of neurons in the brainstem: serotonergic neurons in the MRN and catecholaminergic neurons in the RVM[124]. The PAG in turn receives input from the thalamus, hypothalamus and cortex as well as from the STT and the dorsal raphe nucleus, which mediates some of the effects of PAG stimulation[148]. From the MRN, raphespinal fibres descend bilaterally within Lissauer's tract and terminate in the substantia gelatinosa at all spinal levels where they release serotonin and noradrenaline, respectively. This leads to activation of enkephalin containing interneurons in the dorsal horn, which then excites inhibitory internuncials in the posterior grey horn and blocks nociceptive transmission[137,145]. Additionally, the diffuse noxious inhibitory system-sub-nucleus reticularis dorsalis (DIS-SRD) regulates nociceptive signals as they enter the spinal cord and as they are processed in the brain stem[146,150]. Here, analgesic signals are generated by the MRN in response to input from the spinoreticular and trigeminoreticular neuron activation and block the pain before it is relayed to the higher centres of the brain. This contributes to the

prioritisation of nociception relative to other homeostatic demands[135] and accounts for the analgesic effects of electroacupuncture and similar therapies[145].

At rest, the PAG projection to the MRN is under tonic inhibition by inhibitory internuncials in the PAG. The internuncials themselves are inhibited from releasing substance P by opioid peptides such as β endorphins and enkephalins released from the hypothalamic nuclei that terminate in the PAG. In life threatening situations, the PAG is disinhibited by the hypothalamus[146]. In frightful or emotional situations, it can also be stimulated directly by the release of noradrenaline from the brainstem catecholaminergic neurons. This is known as stress induced analgesia[127]. Perhaps the same mechanisms apply to tendon pain and its disappearance with warm up may be an anticipatory disinhibition of the PAG in readiness for sporting performance[145].

1.4.3.2. The Gating Mechanism

Injury does not always lead to pain perception[151]. Therefore, the CNS has mechanisms that can modify the passage of nociceptive signals and perception of pain. Nociceptive transmission from the trunk and the limbs can be interrupted in the posterior grey horn of the spinal cord and that from the head and upper cervical region in the spinal trigeminal nucleus. The synaptic junctions between the primary afferent fibres, secondary and the tertiary neurons perform a selective action, blocking weak nociceptive signals while allowing passage to, or even, amplifying strong signals. Finely myelinated Aδ polymodal nociceptive fibres synapse directly on the dendrites of relay neurons of the LST but C fibre afferents have indirect access to relay cells via excitatory substantia gelatinosa internuncials. Most of these contain SP, which may

be liberated as a co-transmitter with glutamate[135]. Large mechanoreceptive Aβ afferents from hair follicles also synapse on anterior spinothalamic relay cells and give off inhibitory collaterals, which synapse on lateral spinothalamic relay cells. Some of these also exert presynaptic inhibition upon C fibre terminals. Stimulation of these Aβ fibres through GABA and enkephalins recruits inhibitory gelatinosa cells and decreases C fibre activity and hence suppresses pain[135]. Hence, the nociceptive neurons have their output modulated by input from these non-nociceptive myelinated Aβ afferents. Therefore, if nociceptive C fibre input is greater than Aβ input, then the nociceptive impulse ascends to the brain, otherwise they decay at the spinal cord level[149]. This was initially proposed by Melzack and Wall in 1965 who called it the 'gating' mechanism of pain modification and which can occur at various levels of the CNS[124]. This is the mechanism by which transcutaneous electrical nerve stimulation (TENS) is believed to exert its analgesic effect[149].

The nociceptive and pain pathways are a complex interplay of structures and processes with immense potential for something to go awry and lead to chronic pain[151]. Therefore, with the preceding discussion on the pathological tendon, what is currently known about tendinopathy and the biology of pain, one can hypothesise on potential contributors to chronic Achilles tendon pain.

3.5. POTENTIAL CONTRIBUTORS TO CHRONIC ACHILLES TENDON PAIN

Current theories on the potential sources of pain in tendinopathy have implicated (i) inflammation, (ii) structural tendon changes and (iii) alterations in central pain modulation as possible candidates for the source(s) of pain in chronic AT. Additionally, pain research in other disorders has overwhelmingly shown that genetic polymorphisms also play a crucial role in pain perception. This section will review current knowledge on these causes of tendon pain as well as propose genetic polymorphisms as another potential modulator of the perception of Achilles tendon pain.

1.5.1. Biochemical changes

1.5.1.1. Inflammation

Historically, all forms of tendinopathy were called tendinitis, which implied an inflammatory pathology and automatically an inflammatory origin of the pain. This theory was universally accepted somewhat dogmatically until the late 1970s when histological studies of pathologic tendons demonstrated regions of hyper- and hypo-cellularity, as well as matrix disorganisation with no evidence of inflammatory cell infiltrates[30,31,33,34,152]. Today, the inflammatory hypothesis as a possible explanation for tendon pain is experiencing something of a revival as improved techniques such as immunohistochemistry and gene expression studies have been used to study tendinopathy. As hypothesised by the iceberg theory, inflammatory pathways precede pain in tendinopathy[152], which leads some authors to conclude that without

inflammation, the healing process and changes that characterise tendinopathy would not occur[153,154]. Other authors have even affirmed that chronic inflammation is a feature of tendinopathy[155].

The original studies that discounted inflammation in tendinopathy did so based on the absence of neutrophils only[33,156] and in tendons that had chronic pathology. However, as immunohistochemistry and gene expression analysis techniques have improved, mounting evidence supports some level of inflammation in tendinopathy[33,34,155,157–159] and the definition of inflammation has broadened to include cell types other than neutrophils[33]. Studies have shown that human and equine patients with persistent tendinopathies have increased inflammatory cells counts than those with normal tendons despite having no other histological differences[34,155,159–162]. A handful of systematic reviews also demonstrated that the current literature agrees that there is an increase in inflammatory cells in painful tendinopathy[34,153,163]. However, the authors highlighted the lack of high-quality studies but increased macrophages, T cells, mast cells and leukocytes were observed in the handful of studies selected for review.

Elevated mast cell densities have been observed in early tendinopathy[160,162] and have even been correlated with pain[164]. These mast cells then secrete immune mediators, which then recruit other immune cells via PGE2 signalling. Furthermore, *in vitro*, mast cells stimulate tenocytes to produce increased COX-2 and PGE2, which have downstream effects on the pathology of tendinopathy[165]. Macrophages have also been seen in tendinopathy[160] where they initially adopt a M1 pro-inflammatory phenotype and later change to anti-inflammatory M2 cells. However, in painful tendinopathy, this switch may not occur[162]. Additionally, other studies have shown evidence of B and T

lymphocytes[33,160] and natural killer (NK cells)[166] in painful tendinopathy. The importance of these inflammatory cells is largely in their products, namely cytokines and prostaglandins, which stimulate tenocytes to produce increased amounts of other cytokines and enzymes. Additionally, cytokines and prostaglandins have a direct effect on pain in inflammatory conditions[167–169].

Various studies have noted elevated cytokine and inflammatory mediators such as TNF-α, VEGF, HIF, TGF-β, NF-κB, IL-1β , COX-2, PGE2, and IL-6 in response to mechanical loading and in painful tendinopathy[152,170,171]. These cytokines and mediators are released from lymphocytes, monocytes and endothelial cells and have been implicated in the initiation of the catabolic process of tendinopathy[154,171,172]. For example, elevated IL-21 receptor message and protein has been observed in human supraspinatus tendon cells. This IL-21/IL-21R axis is a cytokine complex that has been associated with confirmed inflammatory diseases such as rheumatoid arthritis (RA) and irritable bowel syndrome (IBS)[173]. IL-17A is also implicated in early rotator cuff (RC) tendinopathy where it is secreted from various immune cells[162,174,175] and is a mediator of tendinopathy. Also, elevated PGE2, COX-2 and TGF-β have been observed in chronic patellar tendinopathy (PT). COX-2 is a major marker of inflammation, which converts arachidonic acid (AA) into prostaglandins (PGs) e.g. PGE2, which is a mediator of pain and acute inflammation in tendons[165,171]. COX-2 is induced by pro-inflammatory cytokines and it in turn induces PGE2 resulting in inflammation[29]. Also, in fibroblasts, TNF-α and TGF-β cause differential regulation of proteoglycans, such as versican and aggrecan, and GAGs, such as chondroitin sulphate[176–180]. These are highly hydrophilic molecules that lead to fluid accumulation in the tendons. This increase in hydrostatic pressure in the tendon acts as a

nociceptive stimulus on the neural sprouts and may cause pain[181]. Other cytokines such as IL-6 have been implicated in inflammation and the initiation of the early phases of tendinopathy and tendon healing[152,155,171]. Furthermore, IL-6 has also been observed in painful AT as well as painful posterior tibialis tendon[182]. More evidence, albeit indirect, of the role of inflammation in this pathology is that tenocyte hyperplasia and hypertrophy is a feature of tendinopathy and tenocytes are known to proliferate and become more metabolically active in response to cytokines and growth factors[33].

These cytokines then lead to pain through the release of SP, glutamate and the production of COX-2 and PGE2[171]. Biopsies from symptomatic Achilles tendons show increased prostacyclin receptors, COX-1, COX-2 and prostacyclin synthase (PGIS). The latter was observed co-localised with cells expressing the glutamate receptor NMDAR-1[183] [glutamate is a known nociceptive neurotransmitter and has now been observed in tendons][31,184]. Treatment of these cells from symptomatic tendons with IL-1β induced prostacyclin metabolites, an observation not seen in normal cells. Additionally, biopsies from individuals whose symptoms had resolved showed increased expression of PGIS, an enzyme, which catalyses the biosynthesis of prostaglandins such as PGE2. These results suggest that PGE2 sustains inflammation in tendinopathy while prostacyclin may be protective[183]. Prostaglandins, prostacyclins and prostanoids contribute to pain peripherally and centrally. Peripherally, they sensitise nociceptors, increase excitability and decrease pain threshold in nociceptors[152].

Another cytokine that has been associated with tendon pain is NF-κB. NF-κB regulates pain in a rodent model of inflammation[185] therefore its role in inflamed tendons could

be postulated to be similar. NF-κB has also been implicated in tendon inflammation and other chronic inflammatory conditions where its activation regulates the expression of over 500 gene products linked to inflammation[171]. In tendinopathy, it is activated by TNF-α and IL-1, which have been demonstrated in tendinopathy as well their complimentary cytokines[162]. Notably, a downstream effect of NK-κB is production of IL-6, which is classically pro-inflammatory as it stimulates T cell formation. The importance of NK-κB in early tendinopathy is further underscored by the observation that 65% of NK-κB associated genes are dysregulated in early stage tendinopathy when compared to healthy controls[155,162,186].

Further, correlations between pain and inflammatory cytokines have been reported in Cher et al's study[187] where they found that patients with idiopathic frozen shoulder had stronger immunoreactivity of alarmins compared to pain-free controls and that expression of the alarmin molecule HMGB1 was correlated with pain. Alarmins are endogenous molecules that are associated with cytokine activation and immune cell function. In addition, in a cohort of individuals with supraspinatus tendinopathy, patients with CD206 and ALOX15 mRNA were pain free following sub-acromial decompression compared to those that did not present these mRNA[155].

Further evidence of the importance of inflammation in tendinopathy is seen in recent associations between adiposity, diabetes mellitus and the risk of tendinopathy[188–192]. The working hypothesis here is that in individuals with high adiposity and/or insulin sensitivity, there is a systemic, chronic, low grade inflammation, which is represented by elevated plasma levels of TNF-α and IL-6, which have a negative impact on tendon health[193–195]. In type 2 diabetes mellitus (T2DM), this low-grade inflammation has been

associated with FOXO1 which is a key regulator of IL-lβ, a pro-inflammatory cytokine that is physiologically inhibited by insulin[29]. This relationship between the low-grade inflammation of obesity demonstrates a role of inflammation in tendinopathy.

Additional evidence for the importance of inflammation in tendon pain is in the efficacy of some treatments. For instance, corticosteroids have been shown to be highly effective in managing tendon pain in the short term[196–199]. Others may argue that corticosteroids are effective because they reverse the pathology however, it is unlikely that they do as the time frames between administration and relief are too short[200–202] and rather suggest an effect on inflammation[33]. Corticosteroids inhibit neutrophils and synthesis of inflammatory cytokines[162]. In addition, their effect on symptoms in tendinopathy are similar to those seen in RA, an established inflammatory condition, which suggests that on some level, the pain of tendinopathy has inflammatory components[152]. Also, some studies have shown positive results with platelet rich plasma (PRP)[199,201,203] whose mode of action has been demonstrated *in vitro* to be anti-inflammatory by regulating the secretion of IL-6, IL-8, and IL-1R[204]. Other treatments such as extracorporeal shockwave therapy (ESWT) have also been successful in some tendinopathy cohorts[205] and *in vivo* investigations have shown that the biological mechanisms of this treatment modality are inflammatory[206]. The extent to which the efficacy of PRP and ESWT can be relied upon as evidence of inflammation in tendinopathy is questionable though as some studies have shown no effect on patient outcomes following treatment with PRP in proximal hamstring tendinopathy[207], AT[208] or PT[209]. Similarly, some studies show no effect of ESWT compared to placebo or other treatments on AT[210–212].

On the other hand, there is some evidence, albeit not recent, to dispute the importance of classical inflammation in tendon pain. Some studies have not demonstrated inflammation in biopsies of AT[213] or the inflammatory response that follows exercise[214]. Also, some murine models have failed to observe evidence of inflammation despite observing other changes associated with tendinopathy[215]. It is also noteworthy that non-steroidal anti-inflammatory drugs (NSAIDS) have disappointingly low success rates in tendinopathy. A double-blind study using piroxicam against a placebo showed no difference in pain rating after four weeks[216]. However, a systematic review on the efficacy of oral NSAIDs in RC tendinopathy found them to provide short term pain relief when compared to placebo and that they were as effective as corticosteroids in the short term[198]. They are however not effective in the medium to long term[217]. These clinical observations are corroborated by results that show no significant increases in PGE2 concentrations in chronically painful tendons[184,218]To be fair, inflammation is unlikely to be the primary cause of pain in chronic tendinopathy. More likely, through cytokine secretion, the inflammatory cells initiate the events of tendinopathy that later lead to pain. Therefore, NSAIDs as an intervention would be more efficacious in the acute stages of the condition[31,33]. However, in addition to recognised gastrointestinal adverse effects, they also have adverse effects on inflammatory matrix components in the tendon[219], which makes them a rather unattractive treatment option for athletes. It is worth noting that most anti-inflammatory medications employed in tendinopathy are COX inhibitors, which target classical inflammation. Perhaps other inflammatory pathways such as neurogenic inflammation could be responsible for the inflammation.

1.5.1.2. Neuropeptides and neurotransmitters

This hypothesis is based on the finding of non-neuronal production of traditionally neuronal nociceptive ligands and agents of neurogenic inflammation in pathological tendons[220]. The most common ligands are glutamate, SP, calcitonin gene related peptide (CGRP) and acetylcholine (Ach)[87,218,221,222]. Glutamate is a potent neuromodulator of pain in the central nervous system and has been widely found to be elevated in painful Achilles, supraspinatus, patellar and extensor carpi radialis brevis (ECRB) tendons where it is suspected to be involved with the pain signalling there too[31,34,37,156,184,223–225]. When compared with individuals whose pain resolved, patients with persistent RC tendon pain have more metabotropic glutamate receptor 2 (mGlut2)[34], and vesicular glutamate transporter 2 (vGlut2) receptors have been detected in tenocytes from AT and PT[226], which suggests that glutamate is active in the pain process.

Glutamate has also been observed to modulate pain in other chronic pain states[87]. In tendons it most likely comes from the abnormal tenocytes themselves[170,220] then activates pain signalling in Aδ and C nerve fibres through its preferred receptor NMDAR-1[227]. Consequently, studies have demonstrated the co-localisation of glutamate and the receptor NMDAR-1 in nerve fibres of painful tendons[37]. This suggests that a peripheral neuronal NMDAR-1 activation by glutamate is involved in nociception. More so since NMDAR-1 is known to trigger a variety of chronic pain disorders and administration of NMDAR-1 antagonists has been shown to decrease pain in arthralgia[37,228]. Contrary to these findings, other research has shown that even after a successful regimen of eccentric training, patients with AT continued to have

higher glutamate concentrations than controls despite them being pain free[229]. In which case, perhaps the glutamate was not their only source of pain as NMDAR-1 requires glycine as well for activation[87] or the NMDAR-1 receptors are also mechanosensitive and where desensitised by the eccentric training or other as yet unexamined substances are responsible for the pain.

Other neuromodulators that have been associated with pain in tendinopathy are SP and CGRP. They (and the preferred SP receptor NK-1R) have been identified in nerve fascicles of neovessels in tendinopathy[33,38,87,152]. SP in particular has a well-known nociceptive role in the central nervous system[220] and is strongly expressed secondary to mechanical loading and in clinical tendinopathy models[230,231]. It is produced by sensory nerves and abnormal tenocytes then modulates the expression of other cytokines[220]. Various studies have shown that SP and NK-1R expression are increased in painful tendinopathies[220,232,233] as are SP positive nerve endings[234,235]. As it is observed mainly in free nerve endings, it most likely plays a nociceptive role[38]. Together with CGRP, it sensitises the nerve endings and reduces the pain threshold[28]. This makes the nerve endings highly susceptible to eventual pain propagation[222]. Indeed, the amount of SP in the sub acromial bursa in patients with RC tendinopathy was correlated with pain and activity limitation in a murine model[231]. The spatio-temporal sequence of SP production is still largely unclear. However, indications are that it is more likely a cause of pain in tendinopathy as it is observed prior to the tendinopathic changes[222]. Additionally, SP has a permissive effect on glutamate[87]. It unblocks the active site of NMDAR-1 by displacing Mg^{2+}, which would otherwise block it. Research on CGRP is in its infancy but thus far it has been shown to potentiate the effects of SP[152].

ACh is also produced by abnormal tenocytes and is capable of modulating nociceptive input[236]. The M_2 muscarinic ACh receptors have been found on tenocytes, nerve endings and neovessels in abnormal tendons particularly in those that are therapy resistant [237–239] and may be involved in a cholinergic anti-inflammatory pathway. Collectively, these results suggest a neurogenic source of tendon pathology and pain, which is worth noting for future studies or development of treatment paradigms.

Although future research is required, it is evident from the preceding section that inflammation, classical or otherwise, plays a key role in tendinopathy. Current evidence does however suggest that this inflammation leads to structural changes in the tendon, which may present a rather obvious source of pain in tendinopathy: the tendon itself.

1.5.2. Structural changes

Proponents of the theory claim that the structural changes such as separation of the collagen fibres and the alteration in the tendon milieu may lead to pain[109]. As described in *Section 1.2.2,* normal tendons have uniformly arranged fibres, are brilliant white and relatively avascular while pathological tendons are grey or brown in appearance with fragile and loose textured tissue[109]. Histologically, areas of hyper- and hypo-cellularity are observed, collagen fibres are disorganised, separated and degenerated and there is an increase in ground substance consisting of proteoglycans and GAGs[68,240–242]. The nuclei of pathologic tenocytes are not aligned parallel to each other and there are variations in orientation of the collagen fibres[67,109,243]. The peritendinous tissue is

thickened and there is hypervascular patterning with proliferation of capillaries and arterioles[244].

In clinical practice, these changes in the architecture of the tendon are visualised using imaging modalities such as magnetic resonance imaging (MRI), grey scale ultrasound (US) and colour Doppler (CD)[13]. In particular, US and CD are commonly used as they are relatively ubiquitous, cost effective, allow for dynamic examination and are even mobile[245,246]. On MRI, problematic tendons present with an increase in signal intensity on T2 weighted imaging sequences as well as thickening of tendons[69] while on US, abnormal tendons tend to have localised widening with local hypoechoic areas. The fibre structure is irregular and neovascularisation can be observed with CD[241]. In addition, pathological Achilles tendons may have convexity on the anterior border[69]. Both the histopathological and imaging findings have been associated with pain in tendinopathy in a number of, but not all, cohorts.

Studies have shown that painful Achilles tendons have decreased expression of versican mRNA, increased expression of type 1 collagen α1 mRNA[247]as well as increased aggrecan and bigylcan mRNA expression[248]. Furthermore, elevated versican content was demonstrated in painful PT[249]. Clinically, greater sulphated GAG content, particularly aggrecan, is increased in pathological tendons[250] and was correlated with VISA scores in PT[181]. Pathology in the tendon results in the progressive disruption of the tendon structure, which may allow the diffusion of these GAGs, which then leads to sensitisation of nociceptive afferents. Alternatively, increased water content in the tendon due to increased GAG content and proteoglycan content[251,252]

may result in higher pressures, which may cause pain. The latter explanation may also explain the MRI signal observed in pathological tendons[215].

A number of imaging studies have explored the relationship between tendon structure and reported pain. Some authors have shown that hypoechoic, heterogeneous and thickened tendons are painful[35,111,253], that the volume of hypoechoic lesions in AT was correlated with VISA-A scores[254] and, in a cohort of volleyball players, that painful patellar tendons presented with more abnormalities on US[255]. Additionally, patellar tendon strain as measured by US was reduced in individuals with PT and this was associated with higher pain report[256]. Research has also demonstrated that as AT symptoms resolved, MRI enhancement was reduced and tendon morphology improved[257]. Combined, these results suggest that perhaps structural abnormalities may be, at least in part, related to tendon pain and as such US changes can be used as prognostic markers to predict future pain and in treatment[35,258]. Accordingly, a number of authors have demonstrated the prognostic value of these US changes; the more heterogeneous the ATs were at baseline, the longer symptoms persisted in one study[259]. Additionally, abnormal tendon imaging findings increase the risk of volleyball and football players developing tendon pain within a season[260–262] and even after three years[263]. Conversely, tendons that were normal at baseline were likely to remain pain free as the seasons progressed[264].

On CD, neoangiogenesis and neovessels have been observed in chronic painful tendons[36,265] with prevalence ranging from as low as 6%[266], 97%[267] and even 100%[268,269] in lower limb tendinopathies. Its chronicity contradicts earlier and current assertions that angiogenesis is a sign of healing and should instead be viewed as a

failed attempt at healing[268,270]. Numerous studies on lower limb tendinopathy have shown an association between increased tendon pain and the occurrence of neovascularisation[271–273]. In fact, it has been widely reported that sites of patient reported pain, induced pain, clinically palpated tenderness and increased CD signal are anatomically related in the Achilles and supraspinatus tendons indicating a relationship between tendon pain and neurovascular changes[36,272,274,275]. The presence of neovessels is associated with pain in PT[276], ECRB tendinopathy[277], in AT[278] as well as in lateral epicondylalgia(LET)[279]. Additionally, the volume of blood flow in these vessels is associated with VISA-A scores in AT[112] and the ablation of neovessels led to improvements in pain in athletes with tendon pain[257,277] while neovascularisation at baseline predicted tendon pain six and 12 months later in AT[280].The expression of hypoxia inducible factor α (HIFα) and vascular endothelial growth factor (VEGF), key angiogenic factors, is also increased in painful tendons[36,281] as is deep microcirculatory blood flow[265]. Hence, the existence of neovessels in pathological tendons should be interpreted as a sign of a persistent and failed healing attempt by the tendon[270]. This underscores the importance of neovascularisation in tendinopathy.

From these data, the working hypothesis is that the neovessels themselves and more likely the nerves that accompany them, are key characters of the tendon pain mechanism[271]. Consequently, most of these neural ingrowths are positive for SP and CGRP, substances that are highly associated with pain[220]. As a result, sclerosing treatments have been proposed as a potential therapy in tendinopathy[272,282]. These treatments work by destroying the nerve ingrowths that are on the blood vessels. To their credit, sclerosants such as polidocanol injections have shown excellent short to

long term success in reducing tendon pain and improving tendon function in PT, AT and ECRB tendinopathies[272,274,275,282–286]. This response to treatment suggests that the blood vessels and not the blood flow in them may be the source of pain[276].

However, contrary to these findings, data from AT and other tendinopathies have not demonstrated this association between structural tendon changes and pain[287,288]. Some authors have noted absence of neovessels in painful tendons[289], and that some painful tendons are normoechoic[254]. Others have shown that 'normal', pain free tendons also have ultrasound abnormalities such as increased thickness, hypoechogenecity and neovascularisation[13,290,291]. In intervention studies, in spite of improvement of symptoms, tendon structure was unchanged following 16 weeks of rehabilitation[13,292] and even though VISA-A scores improved after a 24 week intervention in another study, US and MRI findings remained the same[113]. Similarly, PT symptoms resolved after a four-week exercise intervention but without changes in tendon structure[12] and vice versa in an AT cohort[293].

In other studies, US findings at baseline were not associated with clinical outcome after one year follow up[254] and while Richards et al[257] showed imaging changes with symptom resolution, the imaging findings lagged behind the clinical presentation. This would suggest that tendon structure normalisation is not necessary for the abolition of pain and that structural changes are not required for a positive clinical outcome, hence the two are possibly unrelated. Furthermore, collagen disruption as estimated by peak spatial frequency radius (PSFR) was not related to shoulder pain in individuals with subacromial pain syndrome[294]. With regards to neovascularisation, no relationship was observed between pain scores and neovessels in a number of studies[267,287,291,295–297]. And, while highly encouraging, the excellent results with sclerosants have not been

repeated by other authors outside Scandinavia or with large groups[298]. This has led some authors to conclude that neovessels have no prognostic or diagnostic value[299] and that imaging findings cannot be relied on as a predictor of clinical outcome in tendinopathy[300]. Other authors have gone further to suggest that neovessels serve a biological purpose and should therefore not be eradicated in the management of tendinopathy but rather stabilised to encourage healing[270]. The biological basis for this assertion is that abnormal tendons develop neovessels as a survival mechanism against prolonged hypoxia. Hence, it is rather counterintuitive to expect positive results by going against the body's innate repair mechanisms by destroying the neovessels[270,301]. Further, anti-angiogenic treatments in conditions with a similar neovascular pattern to AT have shown that these treatments actually worsen the condition[301,302].

There are criticisms to using changes in tendon structure to explain tendon pain. For example, the separation of collagen fibres does not necessarily lead to pain as observed in individuals who have anterior cruciate ligament (ACL) repairs using patellar tendon autografts[303] who do not experience PT in spite of collagen excision and lingering abnormalities. In addition, structural changes in tendons are present even in the absence of pain. Studies on asymptomatic patellar tendons have shown that from 14-73% of tendons that have abnormal collagen on US may be pain free when followed up for 12 months[109]. Also, counterintuitively, patients with complete RC tears (more collagen disruption) had less pain than those with partial tears[304].

Furthermore, structural changes on imaging are not a consistent finding hence their clinical utility is questionable[13,305,306]. Some painful tendons are devoid of

neovascularisation and the latter does not in itself guarantee tendon pain as some highly vascularised tendons are perfectly fine[266,271,291,296]. In such patients, perhaps other mediators of pain such as SP, CGRP, glutamate and ACh may be driving the pain mechanism as described in the previous section. These have been traditionally associated with pain in the central nervous system but have been recently identified in tendinopathic tendons[218,307] as well as pain free tendons[266]. This is a plausible premise as angiogenesis is often accompanied by neurogenesis (and these substances are associated with neurovascular ingrowth)[307]. This increases the level of these neural modulators in the tendon, which may stimulate the α adrenergic and NK-1R receptors on the accompanying nerves and elicit pain. This neurogenesis has also been shown to disappear as the tendon heals[34]. Another criticism to using structural changes to explain tendon pain is that due to a dearth of longitudinal studies on tendinopathy, the cause-effect relationship between the symptoms of tendinopathy and its physical features are still largely unexplained.

Yet another major limitation of imaging findings is the variability in machine settings across the studies, the relative subjectivity of the analyses and operator characteristics makes direct comparisons challenging and may well explain the differing results[114]. This is further confounded by the lack of consensus on what constitutes normal, abnormal or pathological tendon ultrasounds[35,114]. This calls for a standardisation of protocols in order to allow more accurate comparisons[305,308]. Additionally, perhaps the technical limitations of the imaging modalities themselves may be the reason why some authors report findings that others do not. This would require the development of more sensitive techniques. However, thus far, even with emerging modalities such as contrast enhanced ultrasound (CEUS) and ultrasound tissue characterisation

(UTC), the relationship between tendon structure and pain is still unclear with conflicting evidence available[112,295].

It appears that tendon inflammation occurs too early to be the direct and only cause of tendon pain and the structural changes, which follow it are not reported consistently hence they cannot explain the pain syndrome in those who do not have them. Perhaps the cause of pain is, at least in part, further afield of the tendon itself and reflects abnormality in not just the tendon tissue but the body's central pain management systems.

1.5.3. Altered central pain modulation

As discussed earlier, tendinopathy has for the most part been considered as a peripheral condition whose pathology (and hence treatment) is confined to itself and its immediate relations. As reviewed in *Section 1.5.2*, there are limitations to pain models that assume a peripheral, tissue-based source of tendon pain. Taking into account the evidence presented to date, it therefore appears that other factors that are further afield from the actual tendon, such as peripheral and central sensitisation, could be involved in enhancing the nociceptive drive from damaged tendons hence causing more or less pain than can be accounted for physically[39,40,87].

As reviewed in *Section 1.4.2.,* general pain begins as a noxious stimulus from the environment, which is detected by the nociceptors and is propagated through tracts up the spinal cord. In specific nuclei of the midbrain and brainstem, the nociceptive

signal is modulated in a process that is catecholamine dependant. Failure or exhaustion of this mechanism may lead to spontaneous or unremitting pain[309,310].

When pain is prolonged, continuous and tissue is damaged, abnormal peripheral and central nociceptors pathways are facilitated and developed. Peripherally, salient nociceptors are activated and in addition some of the products/causes of tissue injury also alter the physiological properties of the nociceptors. They begin to initiate signals spontaneously, which leads to self-generating chronic pain and decreased endogenous pain modulation[126,142,149]. Prolonged C-fibre stimulation alters the pattern of gene transcription in the DRG and dorsal horn cells. This causes increased expression of pain related neurotransmitters in the central nervous system. As a result, non- noxious stimuli become painful (allodynia) and noxious stimuli are met with an exaggerated response (hyperalgesia)[139,149]. The result is an increased rate of firing of the peripheral nerves, which amounts to an increased sensory input to the CNS. This phenomenon is called *peripheral sensitisation*. The persistent barrage of signals to the CNS results in long term changes in neuronal activity in what is known as *central sensitisation*. This has been observed in studies of chronic painful conditions including tendinopathies[311].

Additionally, sustained peripheral nociceptive stimuli from the injured structure may sensitise the nociceptors to an extent that it counteracts the effects of descending inhibition, which would lead to dysfunctional endogenous analgesic control and hence altered pain perception[127]. It is hypothesised that the ability to modulate descending pain may be altered in patients who are susceptible to and develop chronic pain syndromes[123]. The integrity of the facilitatory and the inhibitory systems can be

assessed by experimental conditioning pain stimuli, e.g., cold pressor or ischaemic pain, and assessed by another phasic test stimulus. This is called conditioned pain modulation (CPM)[312]. A reduced CPM implies low capacity of endogenous pain control and can be used as a biomarker of pain or predictor of clinical outcomes[313,314] and is associated with higher pain morbidity[315].

The involvement of the CNS in longstanding pain is supported by evidence from other painful conditions, which has implicated the CNS as a driver of chronic pain[313,316,317]. Altered CPM as well as altered central pain processing have been demonstrated in endometriosis[318], chronic low back pain (LBP)[319–323], temporomandibular disorder (TMD)[324], fibromyalgia (FM)[325,326], rheumatoid arthritis (RA)[327], osteoarthritis (OA)[328], chronic pancreatitis[329], IBS[330], and, chronic widespread pain[331]. In these conditions, as with AT, physical damage does not often match with pain report from the patients and therefore centralised pain may explain the pain behaviour[332].

Evidence of the involvement of the CNS in tendinopathy, in particular of the upper limb, is widely available[40]. The role of CNS involvement in RC pathology has been explored[333]: lower pressure pain thresholds (PPT) were observed in all muscles tested in individuals with shoulder impingement syndrome when compared to pain- free controls[334]. Central sensitisation has also been observed in individuals with carpal tunnel syndrome (CTS)[335] and LET[133,336]. Furthermore, cortical motor reorganisation occurs as a result of chronic RC pathology[337]. Additional evidence for a role of the CNS in upper limb tendinopathies is seen in that unilateral LET pain leads to bilateral sensory changes and sensitisation[221,338]. Furthermore, bilateral sensorimotor

dysfunction has been observed in other cohorts of LET as well as in various tendinopathies[339].

CNS involvement in lower limb tendon pain was demonstrated in chronic patellar tendinopathy[340,341], AT[39] and Debenham and colleagues[342] showed that chronic Achilles tendon pain leads to somatosensory cortex re-organisation as observed by impaired tactile acuity in the unaffected limb in individuals with AT compared to controls. Other studies have also shown increased muscle sympathetic nervous system nerve activity in AT[343] and patellar tendinopathy[344]: upregulation of the sympathetic nervous system (SNS) is a known contributor to chronic pain via the peripheral nervous system (PNS) and CNS. Additionally, 25% of individuals with recalcitrant tendinopathies are thought to have some form of central sensitisation as a characteristic of their pain profiles[345]. A recent elastography study also showed that patients with unilateral Achilles tendon rupture had degenerated contralateral tendons[346]. As a whole, in addition to considerations of loading in the lower limb, these results also imply widespread hypersensitivity, which implicates the CNS in the pain process.

However, other work has shown conflicting results with the role of the CNS in tendinopathy not consistent but rather dependent on the tissue type[343]. Additionally, though local pressure pain thresholds were reduced, there was no evidence of central sensitisation in PT and AT in a study by Plinsinga et al[132] and central sensitisation is observed inconsistently in lower limb tendinopathies[347]. Though the phenomenon is all but set in the upper limb, inferences in the lower limb should be done with caution as there is a dearth of studies on the subject especially on AT[40]. These inconsistencies

in the data are not unique to tendinopathy; the evidence for central pain mechanisms in other chronic pain syndromes is also equivocal. Altered descending pain mechanisms have been observed in some LBP cohorts[331] but not in others[348]. What is constant however, is that individuals with chronic pain in a particular area of the body also tend to have generalised alterations in pain sensitivity[327]. As this is a hallmark of descending control from the brain, this suggests that at some point, chronic pain ceases to be confined to the initially pathological structure and centralises.

What is clear from the evidence is that chronic pain is associated with alteration in the central pain mechanisms. Notably, it has some effects on the hypothalamo-pituitary axis (HPA) and on the limbic system[349–351]. Initially, pain is sensory-discriminative input from the nociceptors and involves minimal cognitive processing. As it persists there is an affective component to its long-term implications. Ultimately, behavioural changes and activity modification/avoidance occur in response to pain[352]. Therefore, in chronic pain, tendon pain notwithstanding, there are aberrant peripheral pain signals, abnormal pain pathways in the CNS and psychological mal-adaptations and behaviours in response to the unremitting pain[353]. These emotional aspects of pain are encoded by corticolimbic systems including the HPA axis, which relates pain, memory and mood. The same systems contribute to the symptomatology of depression, which is a common feature of chronic pain[354]. This psychological effect of chronic pain has also been observed in chronic Achilles tendon pain[99,100]. Hence, while the precise mechanisms are still elusive in AT, it is likely that the CNS to some extent generates, exacerbates, or maintains chronic AT pain. This has clinical and research implications as other therapeutic paradigms can be considered in the management of

chronic Achilles tendinopathy such as treatments, which target the descending inhibitory system[355].

The preceding section presents a body of evidence that suggests that chronic tendinopathy may be associated with changes in the central pain mechanisms. However, some variability in this presentation is still observed, especially in lower limb tendinopathies. A major cause of biological variability in other chronic conditions is genetic and genetic variability has been widely shown to alter risk of developing tendinopathy and other musculoskeletal conditions. Is it therefore plausible that genetic variation can also potentially explain some of the variability in clinical presentation of chronic Achilles tendon pain?

1.5.4. Genetic variations

The association between certain genetic polymorphisms and susceptibility to tendinopathy, specifically AY, is well established in the literature. Most of the genes that have been implicated encode structural components and regulatory molecules within tendons[42,356–358]. Previous research, which was recently reviewed and will therefore not be reviewed in this thesis, has focused on the association of collagen genes, specifically *COL5A1*[359,360], *COL11A1* and *COL11A2*[361] and *COL27A1*[362], proteoglycans (*BCN*)[363], other structural components (*TNC*)[362,364] and *FBN2*[365], matrix proteinases/proteinase inhibitors (*MMP1)*[366], *MMP3*[367,368], *MMP8*[366] and *TIMP2*[368], apoptotic factors (*CASP8*)[369], cytokines (*IL1B*, *IL1RN*, *IL6* and *IL6R*)[370], and other regulatory molecules (*GDF)*[371] and *MIR608*[372] with various tendinopathies. Genetic variants, which have previously been associated with pain in other MSK conditions

have to date not been investigated in AT and could modulate the inter-individual variability in the pain experiences during AT.

In a first of its kind study of more than 500 genes and more than 4000 single nucleotide polymorphisms (SNPs), Ho et al[373] showed that individuals with knee OA who had a *COL11A2* rs16868943 (G>A) GG genotype had on average a higher heat pain tolerance. *COL11A2* encodes for the α2(XI) chain of type XI collagen and interestingly, a knockout mouse model has also reported an increased risk of developing OA[374]. While *COL11A2* has not itself been associated with OA risk in humans, the *COL11A1* gene, which encodes for the α1(XI) chain of type XI collagen, has been associated[375]. The *COL11A2* gene has been associated with osteochondroplasia[376] and non-ocular Stickler's syndrome[377], both of which share clinical features with OA. It is tempting to speculate that *COL11A2* might not only be associated with risk of developing these conditions but potentially in the development of the associated pain. Interestingly, although the genotypes were not independently associated, the inferred T-C-T pseudo-haplotype constructed from the *COL11A1* rs3753841 (T/C), *COL11A1* rs1676486 (C/T) and *COL11A2* rs1799907 (T/A) polymorphisms has previously been associated with increased risk of Achilles tendinopathy in two independent populations[361]. In addition, the TT genotype of *COL11A1* rs3753841 (T/C) and the inferred T-C haplotype constructed from *COL11A1* rs3753841 and rs1676486 has also been associated with CTS[378]. Since types XI and V collagen are structurally and functionally related[379], *COL11A1*/*COL11A2* and *COL5A1* gene interactions have been investigated and also reported to be associated with Achilles tendinopathy and CTS[361,378] while *COL11A1* has also been associated with elbow tendon pathology[380]. Although not the focus of this thesis, the association of *COL11A1*, *COL11A2* and

COL5A1 polymorphisms with AT pain should be investigated. It would also be interesting to investigate the possible association of other collagen genes and tendon pain.

Individuals with a GG genotype of *MMP-1* rs1799750 on average presented with higher temporomandibular joint (TMJ) pain scores[381]. This finding was replicated in an independent cohort of LBP patients were individuals with the GG genotype also presented with higher pain scores on the McGill Pain Questionnaire (MPQ) as well as more disability[382]. In addition, expression of MMP-10 mRNA was significantly increased in surgical patients with pain compared to pain free controls in a study by Richardson et al[383]. Furthermore, in animal models, MMP-2 expression was elevated in models of neuropathic pain[384]. Additionally, the roles of matrix metalloproteinases (MMPs), in particular MMP-2 and MMP-9, in facilitating inflammatory pain have been demonstrated in various studies were these enzymes are required in the early and late stages of neuropathic pain development[385]. Also, their inhibition led to pain relief[386,387] and decreased mechanical allodynia[388] in rodent models. Future studies should also investigate the possible association of polymorphisms within the family of *MMP* and *TIMP* genes, which have previously been associated with AT[389], and tendon pain.

Another family of enzymes that has been implicated in pain and is of interest in tendinopathy is the caspases (CASP), which are protease enzymes involved in apoptosis and inflammation[390,391]. The literature shows that one member, CASP-6, regulates chronic pain via microglial inflammatory signalling, most likely through TNF-α secretion[390,392]. Additionally, inhibition of CASPs-1, -3, -2, -8 and -9 attenuated pain related behaviour in HIV/AIDS and cancer neuropathy models[391]. This suggests that caspase signalling pathways contribute to pain. This is further demonstrated by an

association between *CASP- 9* (-1263A/G) polymorphisms with low back pain[393]. In this study, carriers of the G allele (GG + AG) were overrepresented in the pain group compared to their pain-free compatriots. Similarly, Guo et al[394] showed an association between *CASP-9* (-1263A/G) GG genotype vs AA +AG with discogenic LBP. Since polymorphisms within *CASP8* have been associated with AT in some[395,396], but not all studies[365,389], future work should also consider it and other *CASP* genes as candidates for association with tendon pain.

Ion channels are very important in the pain pathways, where they manage the generation and processing of pain signals. Sodium, potassium and calcium channels are the main ion channels in neuronal transmission in nociceptors and have been firmly implicated in human pain disorders[397]. In particular, voltage-gated sodium channels 1.7 ($Na_v1.7$) is expressed in nociceptors and amplifies subthreshold stimuli. Unsurprisingly, a number of clinical effects such as primary erythermelalgia, paroxysmal extreme pain disorder and congenital insensitivity to pain have been observed in individuals with gain of function and loss of function $Na_v1.7$ mutations, respectively[398–403]. In addition to these rare diseases that result from high impact mutations, polymorphisms in the gene encoding the alpha subunit of the sodium voltage gated channel 1.7 (*SCN9A)* were also associated with subtle effects in modulating risk and severity of pain in acquired pain conditions. For instance, a variant in *SCN9A* rs6746030 (G>A) results in an amino acid substitution of arginine (R) to tryptophan (W) (A1150W) in the alpha subunit of $Na_v1.7$ sodium channel, which enhances cell excitability of DRG[398]. Some studies have demonstrated that the minor A allele of rs6746030 was associated with increased pain reports in individuals with OA, sciatica and phantom limb pain[399] as well as postoperative pain[404] and small fibre

neuropathy[405]. Additionally, functional characterisation of two novel synonymous variants of *SCN9A,* M1852T and T1596I, demonstrated gain of function changes consistent with an increase in neuronal excitability in a diabetic neuropathy cohort[406]. Similarly, variants in genes encoding $Na_v1.8$ and $Na_v1.9$ have also been shown to contribute to inflammatory and neuropathic pain[407,408] and familial episodic pain[409], respectively. However, other authors have not been able to replicate these results in other cohorts[410]. The potential relevance of Na_v to pain in tendinopathy was demonstrated by results, which reported success with topical lidocaine in managing PT pain[411]. As lidocaine provides pain relief by blocking Na_v channels, their results suggest that peripheral Na_v channels play a role in tendon pain, therefore polymorphisms in sodium channels may well affect the pain profile in tendinopathy.

Variants in genes encoding other ion channels such as voltage gated potassium channels (K_v), voltage gated calcium channels (Ca_v) and transient receptor potential cation channel subfamily V member 1 (TRPV1) have also been associated with chronic painful conditions. A polymorphism in potassium voltage-gated channel delayed rectifier subfamily S, member 1 *(KCNS1)* rs734784 (A>G) was associated with higher pain in healthy controls and in phantom limb pain though the same variant did not have an effect on post mastectomy pain[412]. Additionally, a number of variants in voltage gated, inward rectifying and two pore potassium channel genes were associated with risk and severity of persistent pain following surgery[413,414]. Voltage gated Ca^{2+} channels constitute the main pathway for depolarisation mediated Ca^{2+} influx into neurons and genes that encode these Ca^{2+} channels have been implicated in painful conditions. For example, *CACNA2D3*, the gene that codes for the α2δ3 subunit of the voltage dependent calcium-channel complex, has been associated with

thermal pain-related behaviour in animal studies[415]. In healthy volunteers, the rare C allele of the *CACNA2D3* rs6777055 (A>C) SNP was also associated with reduced acute thermal pain while individuals homozygous for the minor alleles showed reduced risk for chronic back pain post discectomy[415]. Additionally, *CACNG2* variants (*CACNG2* encodes the gamma-2 transmembrane AMPA receptor protein stargazin) affect susceptibility to chronic pain after nerve injury in mice[416]: in humans, an association was observed between increased susceptibility to pain after mastectomy and the A-C-C haplotype of three of its intronic SNPs (rs4820242 (G>A), rs2284015 (C>G), and rs2284017(T>C)[417].

Other ion channels whose expression will likely change in AT are acid sensing ion channels (ASICs). The role of ASICs in pain has been demonstrated in rodent studies where *ASIC* gene knockout mice were resistant to mechanical hyperalgesia[418,419] as well as being linked to migraine in humans[420]. ASICs have also been associated with other painful conditions that involve acidosis, inflammation and ischaemia[87,419].

A number of inflammatory mediators such as cytokines are also important in the pathology of tendinopathy. These cytokines include TNF-α, FGF, NF-κB, TGF-β and the IL-1 family whose members are considered very potent[155]. In particular, IL-1α, IL-1β and IL-33, with a signalling pathway through the activation of mitogen activated protein kinases (MAPKs), stimulate mediators of inflammation causing the onset of pain and ECM breakdown[171]. Research in other chronic painful conditions has shown some associations between cytokine gene polymorphisms and pain. For instance, a study on lung cancer patients showed that certain genotypes for *PTGS2* (the gene that encodes COX-2) and *TNF-α* are protective and permissive to pain, respectively[421]

while genetic variants in *COX-2* (haplotype composed of rs2383515 G, rs5277 G, rs5275 T, and rs2206593 A) were associated with post-treatment pain following endodontic treatment[422]. In a study of women with breast cancer, carriers of the minor A allele of *IL1R1* rs2110726 (G>A) reported less breast pain while those with the minor A allele of *IL13* rs1295686 (T>A) had higher pain scores[423]. Stephens et al[424] also showed that variants in *IL1R2* rs11674595 (T>C) and the *IL10* haplotype A8 were associated with persistent postoperative pain following breast cancer surgery. In other work, Stephens and colleagues also showed that variations in *IL6, CXCL8, and TNF* are associated with the development and maintenance of mild persistent breast pain after breast cancer surgery[424].

A study of Chinese females diagnosed with endometriosis also showed an increased representation of the C allele of rs4778889 (T>C) in the *IL16* gene compared to healthy controls and particularly in those with higher self-reported pain[425]. Results that were not replicated among Iranian women[426]. Individuals carrying at least one G allele of *IL6* rs1800795 (C>G) also reported higher pain scores in the postoperative period following total hip replacement[427]. Additionally, simultaneous carriage of other interleukin polymorphisms, *IL-1RNA* and *IL-1αT* has been shown to increase the pain intensity and duration in chronic LBP[428]. This suggests that *IL-1* gene locus polymorphisms promote or prolong low back pain. Additionally, gene variants in *IL-1α* rs1800587 (G>A) affected pain scores and pressure pain threshold in a cohort of patients with lumbar radicular pain. In this cohort, the T allele was associated with an enhanced promoter activity resulting in increased gene expression, which enhances the release of IL-1α and hence the inflammatory response and pain report[429]. Furthermore, an association between the *IL-1α* (C^{889}-T) polymorphism and pain

intensity was observed in a cohort of middle-aged Finnish men with low back pain[428]. Similarly, Moen et al.[430] also showed that in lumbar radicular pain, patients with the *IL1A* T allele in combination with the *IL1RN* A allele had more pain and a slower recovery than other patients. Bessler et al.[431] did not however show a relationship between postoperative pain or morphine use and *IL-1β* polymorphisms in a cohort of women undergoing transabdominal hysterectomy.

A handful of studies have been conducted on the relationship between interleukin gene polymorphisms and tendinopathy. In one, the AA genotype of *IL6R* rs2228145 (C>A) was associated with reduced risk of developing CTS in a South African cohort[363]. In another, while variants within the *IL-1β*, *IL-6* and *IL-1RN* genes were not independently associated with AT risk, when found with the *COL5A1* rs12722 (C>T) polymorphism, they were collectively implicated in modulating risk of AT[432]. As the association with the condition has been established, it would be of interest to further explore the relationship between interleukin genes and tendon pain.

Available evidence also suggests that tendon pain may be a result of defective central pain inhibition. The catecholaminergic system plays a crucial role in the facilitation or inhibition of nociceptive transmission[148]. Abnormalities in catecholamine physiology are associated with decreased activity of COMT, an enzyme, which inactivates catecholamines. As a result, *COMT,* the gene that encodes the enzyme, is one of the most frequently studied of pain genes and has been associated with differential pain sensitivity under experimental and pathological conditions[433] as well as with anxiety, depression, and other psychological traits that influence the perception of pain[434].

Most commonly, various studies have demonstrated the association between Val/Met substitution at codon 158 in the *COMT* gene and numerous pain disorders[435,436]. Individuals homozygous for the Val variant have three to four times higher activity of the COMT enzyme and hence reduced pain sensitivity compared to homozygotes of the Met genotype[437] whose variation leads to decreased COMT thermostability, activity and increased pain report in experimentally induced pain[434,438,439] and in orofacial pain[436]. Furthermore, polymorphisms in *COMT* rs4680 have been associated with Parkinson's disease (PD) related pain[440], FM pain sensitivity[441,442], depression and experimental pain[434,443] and variability in OA pain[444].

Other *COMT* SNPs such as rs4818 (C>G), rs4633 (C>T), rs6269 (A>G) were also associated with lower pain related disability in LBP in a mixed European cohort; the minor alleles of rs4818, rs4629 and rs4633 were associated with lower baseline disability scores[445]. Carriers of rs4818 CC genotype were also over-represented in a Brazilian cohort of FM[442] and the minor alleles of rs4680, rs4818 and rs4633 were over-represented in other FM cohorts though not associated with pain[446]. *COMT* rs6267 (G>A) has also been associated with PD pain wherein the T allele presented with higher pain scores[447]. In addition, *COMT* rs165774 (G>A) showed an association with heat pain; the wild type GG genotype with lower pain threshold than the mutant type while rs886200 CC carriers tolerated cold pain longer[443,448].

In addition, *COMT* variants have also been associated with chronic post-surgical pain. Lee et al[449] showed that individuals carrying the CC genotype of *COMT* rs4818 presented with lower pain scores than those with the CG and GG genotypes following molar extraction. While the high pain haplotypes A-C-C was associated with severe

pain after breast cancer surgery[450]. Additionally, *COMT* variants have been associated with post-operative analgesic response[451]. In the latter study, minor allele carriers of rs6269, rs4633, rs4818 and rs4680 were three times more likely to require analgesic intervention than homozygotes of the major alleles*. COMT* haplotypes have also been shown to affect response to analgesia in TMD[436]. Some authors have however not replicated these findings[443].

Wang et al[452] found no association between rs4680 and chronic post-surgical pain following caesarean section. Additionally, in other cohorts, there were no associations observed between common *COMT* variants and chronic pain conditions such as PD related pain[447], pain sensitivity in chronic widespread pain[453], chronic LBP[445], pain sensitivity in FM[454], vulvodynia[455] and chronic post-operative pain[456]. The HUNT study was also unable to show any associations between *COMT* genotypes and twelve MSK conditions in a large Norwegian cohort[457]. Moreover, no associations were observed between recovery and pain intensity in a European cohort of whiplash injury[458], migraine headache in a Japanese cohort[459] or pancreatitis in an independent cohort[460]. These conflicting results among studies on the association between genetic and clinical variables may reflect the heterogeneity of pain conditions and the small sample sizes of some of the reported studies. It is important to note that three haplotypes of *COMT* (rs6269, rs4818 and rs4680) account for about 11% of the variability in pain perception and given the polygenic nature of pain perception, this is a substantial contribution[437].This underscores the significant role that COMT likely plays in pain chronification.

Variants in *COMT* have also been associated with depression and other mood disorders[434]. The potential role of the Val/Met polymorphism in pain could be because individuals with the Met/Met genotype have greater activation of the limbic regions (anterior cingulate cortex-ACC) of the brain in response to emotionally challenging circumstances and negative stimuli such as pain[461] and lower activation of the dorsolateral prefrontal cortex[462] when compared to Val/Val. The ACC is a key structure of cortical pain processing that is involved in the affective evaluation of pain as well as anti-nociception[127]. Additionally, Met/Met individuals also have higher pain sensitivity and dysfunctional μ-opioid receptor mediated mechanisms in the parahippocampal regions when challenged with prolonged pain[463]. The latter region has an integral role in episodic memory and emotional pain processing. In addition, *COMT* rs6267 GT genotype was associated with depression and PD pain[440]. Another *COMT* variant that has been associated with cognition is rs4818. The CC genotype has also been shown to have lower efficiency at processing emotionally arousing stimuli[464]. This may predispose carriers to stress and dysfunctional responses in the face of disadvantageous situations such as chronic pain. What is clear from these data is that these *COMT* SNPs may reduce opioid mediated inhibitory control of pain, impact brain activity in cognitive domains and hence alter both the physiological and psychological domains of pain processing in a chronic pain condition. Chronic tendon pain affects mood and general affect in sufferers[99,100], as well as being associated with altered CPM[39], which is a proxy for the internal analgesic system. Since the internal analgesic system and mood are both reliant on COMT, any aberrations in COMT are relevant to the study of chronic tendon pain.

The candidate gene approach to identifying genetic targets of disease, though commonly used (as in the studies cited above) and cost effective, is fraught with challenges. Not least the amount of time it would take to sift through the human genome and study the various polymorphisms in order to identify 'pain genes' in tendinopathy. What could hasten this identification process may lie in heritable elements capable of controlling entire constellations of functionally related genes. This is a quality that is inherent to microRNAs (miRNAs)[465].

MiRNAs are small fragments of non-coding RNA that interact with mRNAs to control protein translation. Though their functions remain largely unknown, they have been associated with many physiological processes, mood and regulation of translation of target genes[466]. They are also highly stable in serum and plasma and under varying pH and temperature conditions, which supports their role as reliable biomarkers[467,468]. In addition, miRNAs regulate genes involved in activation of immune cells and secretion of inflammatory cytokines, which suggests that miRNAs are key contributors to the pain amplification and psychological distress, which are characteristic of chronic pain conditions[469].

Aberrant miRNA profiles have been associated with several animal models of pain and inflammation as well as painful conditions in humans. The miRNAs rno-miR-30d-5p, rno-miR-125b-5p and rno-miR-379-5p were observed in rat models of neuropathic pain[465] and miR-134 is modulated in the trigeminal ganglion in response to inflammatory pain[469]. Additionally, miR-143-3p expression was demonstrated in the blood and DRG tissue of mice with collagen induced arthritis. The target genes of miR-143-3p include the inflammation genes *PTGS2* and *TNF-α* and these, together

with hyperalgesia, were observed to be increased in this model[470]. Other work has also shown that miR-103 simultaneously regulates the expression of the three subunits of the voltage gated Ca^{2+} channel, which is important in the context of chronic pain because sensitisation to pain involves the activation of various types of voltage-gated calcium channels[469].

In other studies, miR-132 blood levels were significantly altered in patients with chronic regional pain syndrome[471]. Further, levels of miR-135a-5p differed significantly between individuals who developed chronic axial pain following a motor vehicle accident and those who did not[472]; miR-135a-5p regulates serotonin receptor and transporter transcripts, which are involved in pain processing.

Furthermore, in a fibromyalgia cohort, miR-103a-3p was correlated positively with pain while miR-320a correlated inversely with pain and miR-374b-5p correlated inversely with pressure pain threshold[473]. Other researchers also demonstrated differential expression of numerous miRNAs in subjects with vestibulodynia plus IBS compared to those with either condition or were healthy[474]. Both vestibulodynia and vestibulodynia plus IBS groups demonstrated dysregulation of miR-485-5p, miR-1294, miR-520f and miR-520D-3p. In the same study, miRNA expression was correlated with pain phenotypes and cytokine levels. Specifically, 12 miRNAs were associated with stabbing pain while one was associated with affective pain and two were associated with pain impact on activities of daily living. Another study of patients with painful peripheral neuropathies observed higher miR-21 and miR-146a expression and lower miR-155 expression when compared to healthy controls[475]. Further evidence of the role of miRNAs in pain is found in a study in healthy volunteers, which showed that miRNAs were differentially expressed following opioid treatment[476].

Collectively, these findings suggest that circulating miRNA could be used to predict persistent pain: shared dysregulation of miRNAs may help to elucidate the mechanisms underlying chronic pain conditions. Additionally, they also suggest that miRNA profiling represents a novel and clinically relevant approach for patient stratification of heterogeneous pain-related conditions such as tendinopathy. This review is by no means exhaustive; however, it does provide a theoretical framework on which to test the hypothesis that genetic variation could be, at least in part, responsible for the variability observed in the pain syndrome of chronic tendinopathy.

In conclusion, AT is a common injury in athletes, which presents as pain and loss of function in the lower limb. The pathology of tendinopathy is seen as changes in tendon structure, but the hallmark of the condition is pain, which is often assessed using the VISA-A questionnaire. However, there are some limitations to its use. In the diagnosis of AT, the anatomical relations of the Achilles tendon should be ruled out as possible sources of pain. Classically, AT pain is of insidious onset, related to loading activity and often resistant to treatment. While the biology of pain in general is well described, some features of chronic tendon pain make it difficult to classify it and hence manage. As a result, there are various hypotheses to explain the source of and behaviour of chronic AT pain. Most commonly, tendon pain is thought to be a result of the structural changes that occur in the tendon as seen on histology or imaging. However, the evidence for this is conflicting with equivocal evidence both for and against the hypothesis. Tendon pain could also be a result of the inflammatory cascades that occur in the pathological tendon especially in the initial phases of the condition. While classical inflammation may not be present in the chronically painful tendon, it is possible that neurogenic inflammation is associated with tendon pain. It is also

possible that altered central pain mechanisms are responsible for the persistence of pain in chronic tendinopathy and evidence in the upper limb is overwhelmingly supportive of this notion. However, data on the lower limb are scarce and conflicting. Evidence from other chronic pain conditions shows that genetic variation explains, at least in part, some of the variability observed in chronic pain conditions. The presentation of chronic Achilles tendon pain is variable and therefore it is reasonable to propose that this variability is influenced by a genetic component.

With this background, the aims of this thesis were to:

(i) characterise Achilles tendon pain using other questionnaires in addition to the VISA-A **(Chapter2)**.

(ii) investigate the relationship between structural tendon changes and self-reported pain in recreational athletes with chronic Achilles tendinopathy **(Chapter 3)**.

(iii) investigate the central pain mechanisms in chronic Achilles tendinopathy **(Chapter 4)**.

(iv) evaluate the relationship between selected gene variants and pain in a cohort of recreational athletes with chronic Achilles tendinopathy **(Chapter 5)**.

1.6. OBJECTIVES OF THE THESIS

1. Describe Achilles tendon pain using multidimensional pain scales; the short forms of the McGill pain questionnaire (sf-MPQ) and Brief Pain Inventory (sf-BPI), as well as the VISA-A questionnaire **(Chapter 2).**

2. Evaluate the relationship between self- reported tendon pain, the grey scale ultrasound and colour Doppler characteristics in chronic Achilles tendinopathy **(Chapter 3).**
3. Evaluate the relationship between conditioned pain modulation and chronic Achilles tendinopathy **(Chapter 4).**
4. Explore and evaluate if variants in genes (*Tac1, NK-1R, COMT* and $Na_v1.7$) involved in the pain pathway are associated with either self-reported tendon pain and/or conditioned pain modulation in chronic Achilles tendinopathy **(Chapter 5).**

CHAPTER TWO

CHARACTERISATION OF ACHILLES TENDON PAIN

A condensed version of this chapter has been published as:

Mkumbuzi, N.S., September, A.V., Posthumus, M., Oulo, B., Mafu, T.S., & Collins, M. (2020) Characterisation of Achilles tendon pain using multidimensional pain scales. *Journal of Science and Medicine in Sport.* **23(3):** 258-263; DOI 10.1016/j.jsams.2019.10.016

2.1. INTRODUCTION

As discussed in **Chapter 1**, tendon pain is often the main reported symptom, the major reason for seeking healthcare and a major patient reported outcome measure following treatment[85,477]. Despite extensive research on the subject, the precise mechanisms or the source of AT pain still remain ill defined[87]. What is clear though, is that Achilles tendon pain is often unresponsive to treatment[32,478], tends to become chronic and persists even after improvement in scores from disease specific outcome measures such as the VISA-A questionnaire.

The most common outcome measure for clinical severity of AT is the VISA-A questionnaire, which assesses how tendinopathy and tendon pain affect physical function in patients[11]. While the VISA-A is a valid and reliable tool in assessing the disease specific aspects of tendinopathy, it does not interrogate Achilles tendon pain beyond the physical domain. This may be a limitation of the questionnaire as studies from other chronic MSK pain conditions have shown that chronic pain affects more than the physical tissues that contain the pathology[479,480]. Chronic pain also has emotive and psychological domains that should be considered in the assessment and eventual management of the pain[331,481,482]. It is possible that one of the reasons for

why the literature is still unclear on the mechanisms of tendon pain is that only one of its domains is studied, the physical. Therefore, to improve the understanding of tendon pain and its management, we ought to explore the different pain domains beyond the physical. One suggestion would include using other assessment tools to complement the VISA-A questionnaire, which assess more than the sensory aspects of the pain and examples include the sf-BPI and the sf-MPQ.

The sf-BPI was originally developed for assessing pain in cancer patients but has since been validated in other non-cancer chronic pain cohorts[483–486]. It is used to assess other aspects of pain such as the effect of pain on an individual's mood and enjoyment/ quality of life[483]. On the other hand, the sf-MPQ assesses the quality of pain by using specific adjectives to describe pain[487]. The sf-MPQ should therefore allow for an increased understanding of the language of pain used by patients experiencing tendon pain, which should assist in deciphering potential differences in pain mechanisms seeing as different pain syndromes are associated with different verbal descriptors[488]. In addition, the sf-MPQ and sf-BPI investigate the perceptual qualities of pain and therefore collectively these pain scales may contribute to an improved understanding of chronic pain[487].

As such, the objective of the study presented in this chapter was to characterise chronic Achilles tendon pain using the sf-BPI and the sf-MPQ questionnaires and to compare the relationship of these other questionnaires with the VISA-A questionnaire.

2.2. MATERIALS AND METHODS

2.2.1. Participants

One hundred and thirty two (132) recreational athletes with chronic AT, mostly marathons runners, responded to a call to participate in the study, which was advertised at running clubs, gyms and runners' groups on social media within the greater Cape Town area (South Africa) from November 2017 to July 2018 (Appendix B). Participation in the study was voluntary and all procedures adhered to the Declaration of Helsinki. Ethical clearance and annual review to conduct the study was obtained from the Human Research Ethics Committee of the University of Cape Town's Faculty of Health Sciences (HREC number 279/2016, Appendix A). Participants signed an informed consent form prior to participation in the study (Appendix D).

For inclusion, the participants with at least one year's experience in their main sport presented with posterior lower limb pain in the Achilles tendon of at least three (3) months duration and at least one of the following diagnostic criteria; (1) early morning pain over the mid-portion of the Achilles tendon, (2) early morning stiffness over the mid-portion of the Achilles tendon, (3) a history of swelling over the Achilles tendon area, (4) tenderness to palpation of the mid-portion of the Achilles tendon, (5) palpable nodular thickening over the affected Achilles, and/or (6) movement of the painful area in the Achilles tendon with plantar-dorsi-flexion (positive ''shift'' test) as described previously[244,489,490].

Participants with a history of current or past fluoroquinolone antibiotic use (within the last 12 months) or previous local corticosteroids injection in the Achilles tendon or the area surrounding the Achilles tendon prior to the onset of symptoms (≤12 months) were excluded. As were participants with less than one year's experience in their main sport, a history of Achilles tendon rupture, insertional tendinopathy, congenital or acquired lower limb deformities and/or surgery in the last two (2) years, on chronic medications, diagnosed connective tissue disorders or any other systemic diseases believed to be associated with tendinopathy[111].

2.2.2. Pain Self-report

After clinical confirmation of chronic AT, the participants completed demographic, sporting and medical history, and pain questionnaires (Appendix E). The participants rated their current clinical severity of symptoms and the impact on physical activity using the self-administered eight question VISA-A questionnaire (Appendix F), which is scored from zero, the worst possible result, to 100, the best possible result.

Participants also rated their current tendon pain on the short form of the sf-MPQ (Appendix G). The sf-MPQ is a multidimensional measure of chronic pain consisting of two main subscales; (1) the 11-item sensory subscale and (2) the four-item affective subscale, which both contain various adjectives used to describe pain subjectively. Six of the items ('aching', 'tender', 'throbbing', 'cramping', 'gnawing' and 'heavy') in the sensory subscale describe continuous pain; four of the items ('sharp', 'shooting', 'stabbing', 'splitting') describe intermittent pain; while one item, 'hot-burning', describes a neuropathic type of pain. The affective subscale has four items: namely, 'sickening', 'punishing-cruel', 'fearful' and 'tiring-exhausting'.

The total pain score for these two subscales ranges from 0 to 45. The sf-MPQ also has a present pain intensity (PPI) score, which consists of six intensity options, giving a score between 0 and 5 and a 100mm VAS score[487]. The final total sf-MPQ pain score ranges from 0 (no pain) to 60 (worst pain).

Finally, participants completed the sf-BPI, a pain assessment tool, which measures pain intensity (four items) and its interference with daily activities (seven items)[491] (Appendix H). The interference items assess the effect of pain on general activity, mood, walking ability, normal work, relations with other people, sleep and enjoyment of life. The intensity items assess pain at its worst, least, average and present over the preceding 24 hours. Each item on the questionnaire is scored 0 to 10[483]. For this study, the scores from the sf-BPI where grouped into none (0), mild (1-4), moderate (5-7), severe (8-10) for analysis.

2.2.3. Statistical analyses

STATA® v15 was used to analyse the data collected in this study. Categorial variables were described as relative frequencies (%). The Shapiro-Wilks test was used to assess the normal distribution of continuous variables. Normally distributed continuous data was represented as means and standard deviations, while medians and interquartile ranges (IQR) where used to describe the variables that were not normally distributed. The Mann-Whitney U test was used for comparative analyses of variables that violated the assumption of normality. Spearman's and Pearson's correlations were used to evaluate correlations between the indices of the three pain scales. The level of significance was set as $p<0.05$.

2.3. RESULTS

Ninety-one (73.4%) participants were primarily endurance runners (n=91) while the rest participated in mainly hiking (n=9), gym (n=4), golf (n=4), triathlon (n=3) or cycling (n=2). Eleven (9.7%) did not specify their main sport. The median (IQR) duration of participation in their main sport was 9 (4 – 20) years. Seventy-six (61.3%) participants were male and the average age of the participants was 44.6 ± 10.7 years, ranging from 18 to 75 years. The median (IQR) BMI was 24.8 (22.9 – 27.8) $kg.m^{-2}$. Eighty-nine (77.4 %), 16 (13.9%) and 10 (8.7%) of the participants were right-legged, left-legged or ambidextrous, respectively.

The median (IQR) duration of symptoms and the VISA-A score on recruitment were 12 (6 – 36) months and 72 (61 – 82), respectively. Most participants presented with Achilles tendon pain on their right side (38.1%, n=45) and a similar proportion had bilateral symptoms (33.9%, n=40). The remaining 33 (28.0%) reported symptoms on the left side. There was no significant difference between the participants' dominant and affected leg (p=0.360).

Tendon pain was largely described using words from the sensory index of the sf-MPQ questionnaire and less so from the affective index (Figures 2.1A and 2.1B, Table 2.1). Generally, the pain was reported to be mild, to a lesser extent moderate and infrequently as severe (Table 2.2). Forty-five (36.3%) selected words that were in only one pain category (continuous, intermittent or neuropathic pain from the sensory scale and affective scale) while 35 (28.2%) selected words in two categories, 28 (22.6%) selected three pain categories and 8 (6.5%) selected from all four categories. Overall,

participants chose either one (n=26), two (n=26), three (n=15), four (n=18), five (n=11) or more (n=20) words to describe their pain. One hundred and ten (88.7%) of the cohort selected at least one word from the continuous pain category ('aching', 'tender', 'throbbing', 'cramping', 'gnawing' and 'heavy') compared to 61 (49.2%) who described it as intermittent ('sharp', 'shooting', 'stabbing', 'splitting'), 30 (24.2%) and 30 (24.2%) who described it as neuropathic ('hot burning') and affective ('sickening', 'punishing-cruel', 'fearful' and 'tiring-exhausting'), respectively (Tables 2.1 and 2.2).

The most frequent descriptor in the sensory index was 'aching' (n=73, 60.3%) followed by 'tender' (n=64, 52.9%) while a third of the participants also used 'throbbing' (n=41, 33.9%), all three terms are descriptors of continuous pain. A third also used 'sharp' (n=40, 33.1%), which describes intermittent pain. Forty-one (33.9%) participants used both 'aching' and 'tender' to describe the pain (Table 2.1), followed by 'aching' used in combination with 'throbbing' (n=27, 23.3%) and 'sharp' (n=27, 23.3%). Eighteen (14.9%) participants described their tendon pain using all three of the most common descriptors, namely 'aching', 'tender' and 'throbbing'. A significant portion of the participants also described the pain as 'tender' in combination with either 'throbbing' (n=24, 19.8%) or 'sharp' (n=24, 19.8%). As summarised in Table 2.1, 'aching', 'tender', 'throbbing' and/or 'sharp' where used less frequently in combination with other pain descriptors from the sensory index (cramping, gnawing, heavy, stabbing, shooting and/or hot-burning) by greater than 10% of the participants. Very few participants (<10%), used descriptor combinations that contained a word choice from the affective index (Table 2.1).

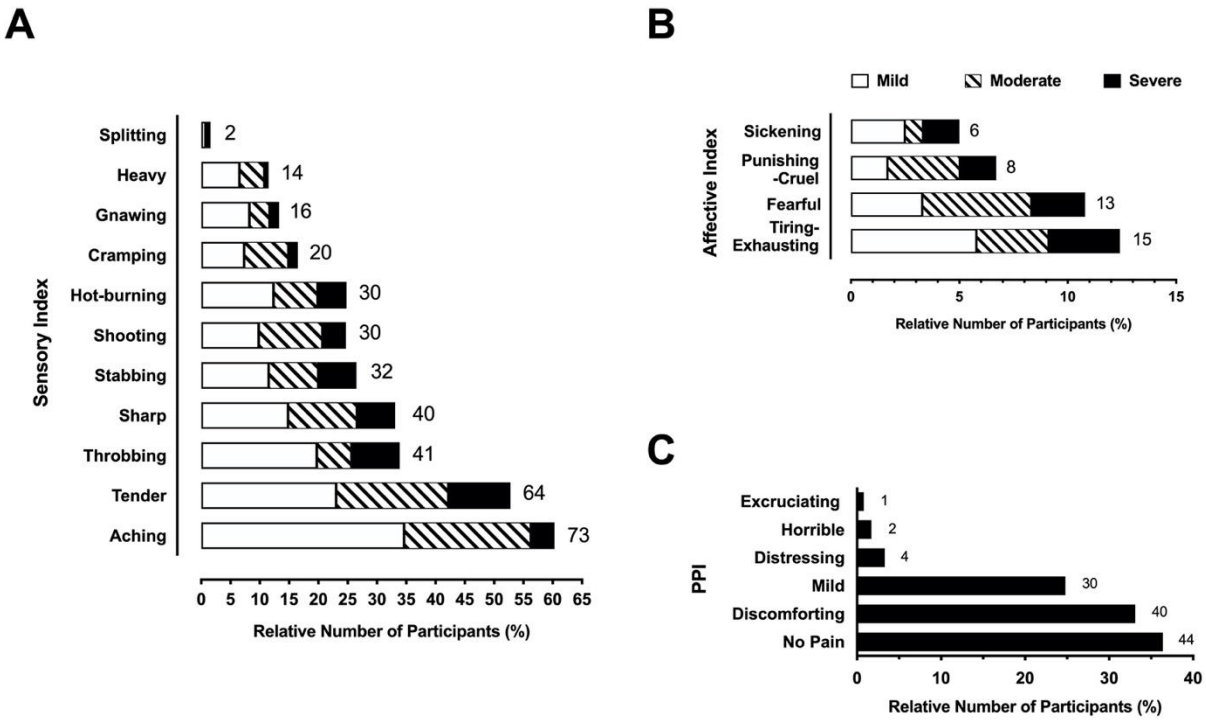

Figure 2.1: Self-reported Achilles tendon pain from the short form McGill Pain Questionnaire (sf-MPQ). The relative number of participants who chose the indicated description words **(A)** from the sensory index of the sf-MPQ, **(B)** the affective index of the sf-MPQ and **(C)** the intensity index as shown on the present pain index (PPI). The number of participants is indicated above each bar. The relative number of participants reporting mild (clear bars), moderate (hatched bars) and severe pain (solid bars) for each descriptor of the sensory and affective index is also indicated.

Three other descriptors of continuous pain, namely ‘cramping’ (n=20, 16.5%), ‘gnawing’ (n=16, 13.2%) and ‘heavy’ (n=14, 11.6%) were identified less frequently (Figure 2.1A). With respect to the other descriptors of intermittent pain, 32 (26.4%), 30 (24.8%) and 2 (1.6%) described their tendon pain as ‘stabbing’, ‘shooting’ and/or splitting, respectively. Thirty (24.8%) participants described their tendon pain as neuropathic; ‘hot-burning’, (Figure 2.1A, Table 2.1 and Table 2.2). The four descriptors in the affective index, namely ‘tiring-exhausting’ (n=15, 12.4%), ‘fearful’ (n=13, 10.7%), ‘punishing-cruel’ (n=8, 6.6%) and ‘sickening’ (n=6, 5.0%), were seldom identified (Figure 2.1B). The median (IQR) pain scores for the two sf-MPQ subscales were 3 (2 – 7) and 0 (0 – 0) for the sensory and affective indices, respectively.

Forty-four (36.4%) of the participants reported no pain on the PPI at the time of testing. Of those with pain, 40 (33.1%) described it as being ‘discomforting’, 30 (24%) as ‘mild’, and four (3.3%), two (1.7%) and one (0.8%) as either ‘distressing’, ‘horrible’ and/or ‘excruciating’, respectively (Figure 2.1C). The modal PPI pain score of the participants was ‘no pain’ (n=44), while they reported median (IQR) pain score on the VAS scale 0.1 (0 – 3) and the total sf-MPQ pain score, consisting of the combined scores of the two sf-MPQ subscales, the PPI and VAS scale, was 7.8 (4 – 12).

Table 2.1: The combinations of descriptor word choices from the sensory [continuous, intermittent and neuropathic (N)] and affective indices of the short form McGill Pain Questionnaire (sf-MPQ) chosen by 10% or more of the participants to describe Achilles tendon pain. The relative number of participants, with n in parenthesis, are also indicated.

Continuous						Intermittent				N	Affective				% (n)
Aching	Tender	Throbbing	Cramping	Gnawing	Heavy	Sharp	Stabbing	Shooting	Splitting	Hot-Burning	Tiring-Exhausting	Fearful	Punishing-Cruel	Sickening	
X	X														33.9 (41)
X		X													22.3 (27)
X						X									22.3 (27)
	X	X													19.8 (24)
	X					X									19.8 (24)
	X						X								17.4 (21)
X								X							16.5 (20)
	X							X							16.5 (20)
			X	X											16.5 (20)
		X				X									15.7 (19)
						X	X								15.7 (19)
X							X								14.9 (18)
X										X					14.9 (18)
X	X	X													14.9 (18)
						X		X							14.0 (17)
X	X					X									14.0 (17)
							X	X							12.4 (15)
X	X							X							12.4 (15)
X	X					X		X							12.4 (15)
X			X												11.6 (14)
	X									X					11.6 (14)
		X					X								11.6 (14)
		X						X							11.6 (14)
		X								X					11.6 (14)
X		X				X									11.6 (14)
X			X	X											11.6 (14)
X					X										10.7 (13)
							X			X					10.7 (13)
X						X	X								10.7 (13)
	X					X	X								10.7 (13)

Table 2.2: Self-reported Achilles tendon pain from the short form McGill Pain Questionnaire (sf-MPI). The relative number of participants, with the n in parenthesis, who chose the descriptor words and the severity from the sensory and affective indices of the sf-MPI. One-hundred and twenty-one (121) participants completed the questionnaire.

Descriptors	Type of pain	None	Mild	Moderate	Severe
Sensory Index					
Aching	Continuous	39.7 (48)	34.7 (42)	21.5 (26)	4.1 (5)
Tender	Continuous	47.1 (57)	23.1 (28)	19.0 (23)	10.7 (13)
Throbbing	Continuous	66.1 (80)	19.8 (24)	5.8 (7)	8.3 (10)
Sharp	Intermittent	66.9 (81)	14.9 (18)	11.6 (14)	6.6 (8)
Stabbing	Intermittent	73.6 (89)	11.6 (14)	8.3 (10)	6.6 (8)
Shooting	Intermittent	75.2 (91)	9.9 (12)	10.7 (13)	4.1 (5)
Hot burning	Neuropathic	75.2 (91)	12.4 (15)	7.4 (9)	5.0 (6)
Cramping	Continuous	83.5 (101)	7.4 (9)	7.4 (9)	1.7 (2)
Gnawing	Continuous	86.6 (105)	8.3 (10)	3.3 (4)	1.7 (2)
Heavy	Continuous	88.4 (107)	6.6 (8)	4.1 (5)	0.8 (1)
Splitting	Intermittent	98.3 (119)	0.8 (1)	0.0 (0)	0.8 (1)
Affective index					
Tiring-Exhausting		87.6 (106)	5.8 (7)	3.3 (4)	3.3 (4)
Fearful		89.3 (108)	3.3 (4)	5.0 (6)	2.5 (3)
Punishing-Cruel		93.4 (113)	1.7 (2)	3.3 (4)	1.7 (2)
Sickening		95.0 (115)	2.5 (3)	0.8 (1)	1.7 (2)

Based on the sf-BPI, eighty-seven participants (72.5 %) reported that tendon pain interfered with their walking ability, with 66 (55.0%) and 17 (14.2%) reporting mild or moderate interference, respectively (Figure 2.2A and Table 2.3). Just over half of the participants also reported that tendon pain interfered with normal work (n=66, 55.0 %),

enjoyment of life (n=65, 54.2 %), general activity (n=64, 53.3 %) and mood (n=61, 50.8 %), which was predominately mild interference (n=55, 45.8%; n=47, 39.2%; n=51, 42.5% and n=41, 34.2%), respectively. Forty (34.8%) of the participants indicated that tendon pain interfered with sleep, 30 (25.0%) indicated interference with relationships with others. Most of the interference was mild (n=34, 18.3% and n=23,19.2%, respectively).

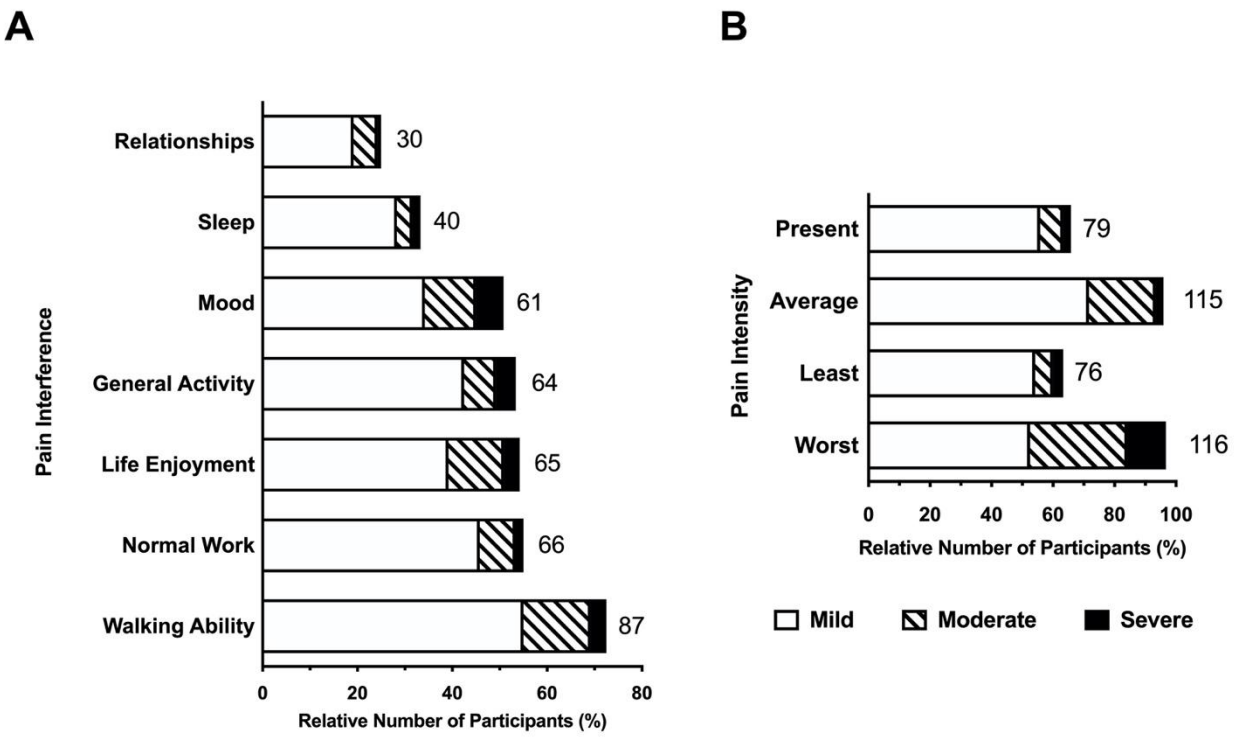

Figure 2.2: Self-reported Achilles tendon pain from the domains of the Brief Pain Inventory (BPI). The relative number of participants and **(A)** the activities that tendon pain interfered with and **(B)** the intensity at its worst, least, on average and at the time of testing. The number of participants is indicated above each bar. The relative number of participants reporting mild (clear bars), moderate (hatched bars) and severe pain (solid bars) for each domain is also indicated.

Table 2.3: Self-reported Achilles tendon pain from the domains of the short form Brief Pain Inventory (sf-BPI). The relative number of participants, with the n in parenthesis, who described the pain intensity at its worst, least, on average and at the time of testing and the activities with which tendon pain interfered. One hundred and twenty (120) participants completed this questionnaire.

	None	Mild	Moderate	Severe
Pain interference domain				
Relationships with others	75.8 (91)	19.2 (23)	5.0 (6)	0.8 (1)
Sleep	67.5 (81)	18.3 (34)	3.3 (4)	1.7 (2)
Mood	50.0 (60)	34.2 (41)	10.0 (12)	6.7 (8)
General activity	47.5 (57)	42.5 (51)	6.7 (8)	4.2 (5)
Enjoyment of life	46.7 (56)	39.2 (47)	10.8 (13)	4.2 (5)
Normal work	45.8 (55)	45.8 (55)	6.7 (8)	2.5 (3)
Walking ability	28.3 (34)	55.0 (66)	14.2 (17)	3.3 (4)
Pain intensity domain				
At present	34.2 (41)	55.8 (67)	7.5 (9)	2.5 (3)
On average	4.2 (5)	71.7 (86)	21.7 (26)	2.5 (3)
At its least	36.7 (44)	54.2 (65)	5.8 (7)	3.3 (4)
At its worst	3.3 (4)	52.5 (63)	31.7 (38)	12.5 (15)

When comparing the feedback related to the intensity of pain at its worst, its least, on average and present pain, most participants (n=63, 52.2%; n=65, 54.2%; n=86, 71.7% and n=67, 55.8%), reported mild pain in all four groups of pain, respectively (Figure 2.2B). Forty-one (34.2%) participants reported no pain at the time of testing (at present) and 44 (36.7%) reported no pain when the pain was at its least, while 38 (31.7%) and 15 (12.5%) of the participants reported moderate or severe pain,

respectively, at its worst (Table 2.3 and Figure 2.2B). Twenty-six participants (21.7%) reported that on average, their pain was moderate (Table 2.3 and Figure 2.2B).

The three questionnaires contain corresponding indices: namely the total (i) sf-MPQ sensory, sf-BPI intensity and VISA-A intensity and (ii) sf-MPQ affective, sf-BPI interference and VISA-A interference scores. There were weak to moderate correlations between corresponding indices on the sf-MPQ, VISA-A and sf-BPI (Figure 2.3). Generally, no sex differences where observed across the pain scales ($p>0.05$). However, from the sf-BPI, tendon pain interfered with various activities more in females than in males ($p=0.034$).

The relationship of six of the commonly used sf-MPQ pain descriptors (Figure 2.1 and Table 2.2) with the participants' VISA-A scores is summarised in Table 2.4 and Figure 2.4A. Although there were no differences in the VISA-A scores when any of the six descriptors were reported as no, mild or moderate pain, the VISA-A scores were lower when participants reported severe tender, sharp and/or stabbing pain (Table 2.4). The median (IQR) VISA-A score was significantly higher in the participants who did not use any of the six commonly used sf-MPQ descriptors to describe their tendon pain [85 (75 - 92), n=9] compared to those who described their pain as severe for at least one of the descriptors [62 (54 - 74), 24; $p=0.002$] (Figure 2.4A). The VISA-A scores were however similar irrespective of the number of different continuous and intermittent pain descriptors the participants chose to describe their Achilles tendon pain. The median (IQR) VISA-A scores when the participants selected none, one or two and all three of the continuous pain descriptors (aching, tender and throbbing) were 77 (62 - 87), n=34; 69 (61 - 78), n=57 and 71 (58 - 78), n= 27, respectively. Similarly, the median (IQR)

VISA-A scores were 73 (65 - 86), n=59; 70 (55 - 79), n=50 and 67 (58 - 78), n=10 when the participants selected none, one or two and all three of the intermittent descriptors (sharp, stabbing and shooting), respectively.

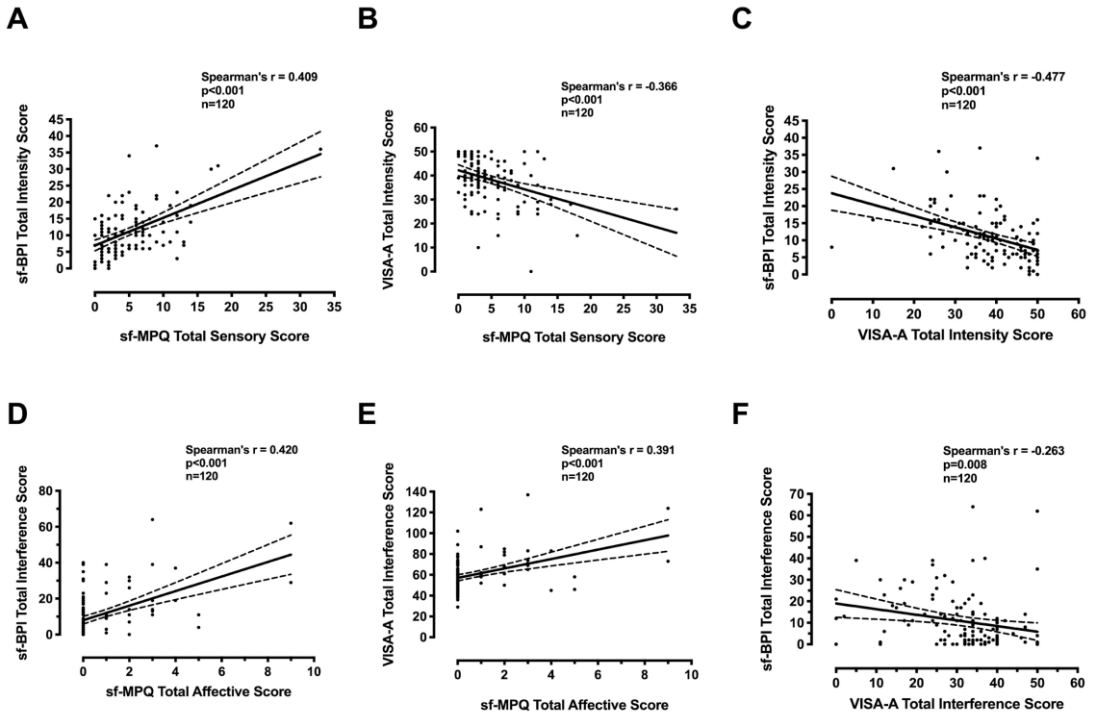

Figure 2.3: Correlations between corresponding indices of the short form McGill Pain Questionnaire (sf-MPQ), short form Brief Pain Inventory (sf-BPI) and Victorian Institute of Sports Assessment - Achilles Questionnaire (VISA-A) questionnaires; sf-BPI total intensity score compared to sf-MPQ total sensory score **(A)**; VISA-A total intensity score compared to sf-MPQ total sensory score **(B)**; sf-BPI total intensity score compared to VISA-A total intensity score **(C)**; sf-BPI total interference score compared to sf-MPQ total affective score **(D)**; VISA-A total interference score compared to sf-MPQ total affective score **(E)**; sf-BPI total interference score compared to VISA-A total interference score **(F)**.

Table 2.4: The median (IQR) values of the Victorian Institute of Sports Assessment - Achilles Questionnaire (VISA-A) of each self-reported Achilles tendon pain severity (none, mild, moderate and severe) for the seven most commonly used pain descriptors within the sensory index of the short form McGill Pain Questionnaire (sf-MPQ).

	None	Mild	Moderate	Severe	p-value
Aching	75 (60 - 86), 48	73 (65 - 78), 41	68 (58 - 81), 25	69 (60 - 76), 5	0.490
Tender	77 (62 - 86), 56	70 (57 - 81), 27	72 (63 - 80), 23	61 (53 - 70), 13	**0.005**
Throbbing	73 (62 - 85), 78	69 (60 - 78), 24	77 (58 - 82), 7	67 (56 - 77), 10	0.240
Sharp	73 (63 - 84), 80	77 (63 - 81), 17	71 (51 - 82), 14	60 (43 - 74), 8	**0.030**
Stabbing	73 (62 - 83), 87	78 (60 - 85), 14	68 (58 - 78), 10	60 (44 - 74), 8	**0.048**
Shooting	73 (63 - 84),89	62 (13 - 74), 16	69 (49 - 75), 13	69 (52 - 80), 5	0.509
Hot burning	73 (64 - 82), 89	61 (56 - 87), 15	71 (44 - 79), 9	70 (47 - 80), 6	0.472

The number of participants (n) in each group is indicated after the median (IQR) values; p-values (none, mild and moderate vs severe). Significant p-values are in bold.

Similar median (IQR) VISA-A scores were reported by the participants for the commonly used PPI scores, namely: none [73 (64 - 82), n=41], mild [72 (60 - 82), n=-26] or discomforting [70 (54 - 82), n=37]. In addition, there was no significant correlation between the participants' VISA-A and VAS scores (Spearman's r = 0.088, p=0.359, n=111).

Additionally, individuals who reported that Achilles tendon pain interfered moderately or severely with all domains in the sf-BPI, except sleep, had significantly lower median VISA-A scores compared to those with no or mild interferences (Table 2.5). Similarly, the median VISA-A scores were significantly higher in the participants who reported that their tendon pain interfered with none or at least one of the domains of the sf-BPI

compared to those who reported that the pain inferred moderately or severely ($p<0.001$) (Figure 2.4B).

Table 2.5: The median (IQR) values of the Victorian Institute of Sports Assessment - Achilles Questionnaire (VISA-A) of each self-reported Achilles tendon pain severity (none, mild, moderate and severe) for the descriptors within the interference and intensity domains of the short form Brief Pain Inventory (sf-BPI).

	None	Mild	Moderate or Serve	P-Value
sf-BPI Pain interference domain				
Relationships	73 (64 - 83), 81	71 (56 - 80), 23	49 (39 - 70), 7	**0.010**
Sleep	74 (65 - 85), 72	68 (54 - 81), 33	70 (61 - 76), 6	0.549
Mood	77 (69 - 86), 53	70 (57 - 80), 38	58 (49 - 76), 20	**0.009**
General activity	77 (68 - 87), 51	70 (57 - 80), 47	61 (44 - 77), 13	**0.042**
Enjoyment of life	77 (69 - 87), 49	70 (57 - 80), 44	62 (49 - 75), 18	**0.011**
Normal work	77 (69 - 87), 49	70 (57 - 80), 52	62 (49 - 75), 18	**0.026**
Walking ability	76 (68 - 88), 30	74 (59 - 80), 60	66 (48 - 77), 21	**0.016**
sf-BPI Pain intensity domain				
At present	80 (70 - 87), 39 [a]	70 (58 - 78), 60 [b]	61 (41 - 73), 12 [c]	**0.002** [a vs b] **0.002** [a vs c] 0.055 [b vs c]
On average	92 (87 - 99), 4	73 (63 - 82), 78	68 (54 - 77), 29	**0.041**
At its least	77 (70 -86), 39 [a]	68 (57 - 80), 61 [b]	61 (53- 76), 11 [c]	**0.004** [a vs b] **0.006** [a vs c] 0.322 [b vs c]
At its worst	88 (83 - 97), 4	74 (68 - 83), 57	66 (53 - 78), 50	**0.004**

The number of participants (n) in each group is indicated after the median (IQR) values; p-values (none, mild and moderate vs severe). Significant p-values are in bold.

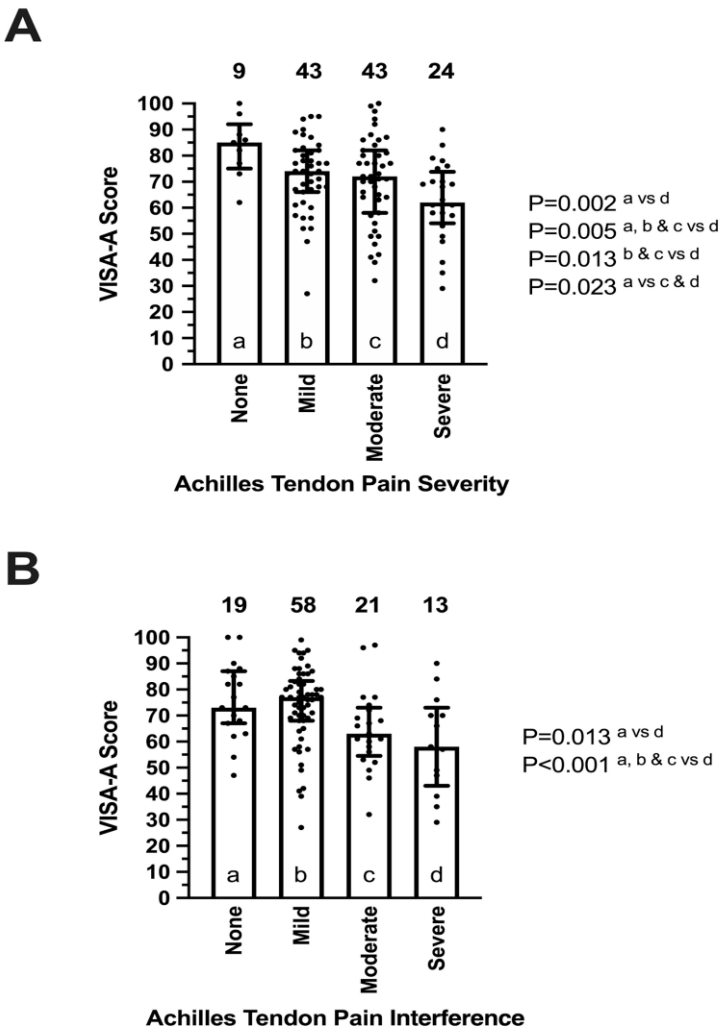

Figure 2.4: The median (IQR) values of the Victorian Institute of Sports Assessment - Achilles Questionnaire (VISA-A) of the self-reported Achilles tendon pain **(A)** severity and **(B)** interference categories (none, mild, moderate and severe) for the six commonly used short-form McGill Pain Questionnaire (sf-MPQ) descriptors and all seven of the short form Brief Pain Inventory (sf-BPI) interference domain descriptors, respectively. Participants who selected "none" to indicate severity for all six of the Achilles tendon pain descriptors were included in the none group in (A). Similarly, those who selected "none" to indicate no interference by any of seven activities were included in the none group in (B). Those who described their Achilles tendon pain severity (A) or interference (B) at the most as mild, moderate or severe for at least one of the descriptors were included in the mild, moderate or severe groups, respectively. The number of participants in each group is indicated above the bars.

Finally, participants who reported that the intensity of the Achilles tendon pain was mild to severe at the time of testing or when the pain was at its least had significantly lower median (IQR) VISA-A scores compared to those who reported none on the sf-BPI pain intensity domain (Table 2.5). However, only those who reported that the intensity of the pain was on average or at its worst moderate to severe had significantly lower median (IQR) VISA-A scores compared to those who reported no (none) or mild pain intensity (Table 2.5).

2.4. DISCUSSION

Previous studies on chronic pain have shown that word choices used to describe pain are significant and are condition specific, examples include cramping for menstrual pain; throbbing for toothache; aching for arthritic pain and tender for post herpetic pain[488]. These adjectives though condition specific are not exclusive and have been used to describe pain in other conditions with various aetiologies and pathologies[488,492–496]. It is therefore valuable to identify descriptors specific to tendinopathy as the cause of pain in tendinopathy is unknown[87] and these descriptors may help shed light on the origin and mechanisms of the pain.

The main, novel finding of this part of the thesis is that from the sf-MPQ, tendon pain was predominantly described as 'aching', which contrasts with current literature where it is traditionally described as 'tender'; both adjectives describe continuous pain. Other descriptors of continuous pain such as 'throbbing' were also employed to a greater extent. In addition, tendon pain was described as an intermittent pain ('sharp', 'shooting', 'stabbing') and as a neuropathic type of pain ('hot-burning'). The description of tendon pain as tender may be in response to palpation; painful tendons *are* tender on palpation[85]. Other researchers have previously used the sf-MPQ in LET[497]. However, these authors did not specify which words were chosen by the participants to describe the tendon pain, which would have been invaluable for comparison with the present cohort.

Each pain syndrome is often characterised by a distinctive group of words and patients show remarkable consistency in these word choices. Word descriptors had diagnostic

value in a cohort of women with various types of gynaecological injury where word choices had high predictive values in ruling out other diagnoses[498]. Different word choices were also used by women with dysmenorrhoea when compared to those with pain from episiotomies[499]. Additionally, word choices have been successfully used to distinguish between trigeminal neuralgia vs atypical facial pain (91%)[500], pulpitis vs pericoronitis[501] and temporomandibular joint pain vs myogenous facial pain[502]. These word choices have also been shown to be surgery specific in individuals with chronic postsurgical pain following various types of surgery[503]. However, in this cohort, the words chosen to describe Achilles tendon pain have also been used to describe pain in other conditions, which have a variety of pathologies. This spread of descriptors across pain types may suggest that there exists more than one source of Achilles tendon pain and even clinical subtypes of the condition[504]. These clinical differences may further explain the variability in treatment response of tendon pain.

The relationship between pain type and treatment outcome has already been demonstrated in LBP where the determination of whether a patient has continuous or intermittent pain has diagnostic as well as prognostic value. Intermittent pain often indicated mechanical LBP, sufferers coped better with the pain and claimed less for workers' compensation[505]. Collectively, these findings demonstrate the need to better describe tendon pain in order to further our understanding as well as management of tendon pain.

Another finding from this cohort was that tendon pain is largely a sensory type of pain and has minimal emotive components. This means that the cognitive appraisal in tendon pain does not lead to fear and avoidance of the inciting/exacerbating activity[100]

as often seen in other types of chronic pain[506]. In this respect, tendon pain differs from other types of chronic pain, which may suggest that despite its chronic nature, tendon pain is largely local/peripheral[132,507] as will be further elaborated on in **Chapter 4**. A possible explanation for this observation could be the main choice of sport of this cohort; endurance running. Regular endurance exercise has been shown to reduce sensitivity to homotypic (more exercise) and heterotypic (other insults such as pain) stressors perhaps as a result of the physical exercise itself or through recruitment of enhanced endogenous analgesic mechanisms[508]. This can be further supported by present total sf-MPQ scores, which were lower than those obtained in other studies of musculoskeletal pain[488] and by the participants' descriptions of tendon pain during assessment. These participants stated that tendon pain interfered with a number of daily activities but only minimally because they, 'don't let it interfere', 'it won't kill [them]' or 'it's more of an annoyance' (Personal communication, 2018). This seems to be a psychological coping mechanism that individuals develop to manage pain in sports participation[509] or occupational pursuits[510]. This trivialisation of pain together with continued sporting activity in spite of the pain does make a case for studying the relationship between tendon pain and personality. This association has previously been identified in other chronic pain conditions[511–513]. Hence, one can hypothesise that the personalities of individuals who tend to get tendon pain may well explain this apparent absence of affective elements of the pain.

Achilles tendon pain is common in endurance runners, a sporting discipline that attracts the kind of personalities who may, as a result of their sport, need to push through pain in order to train for and complete marathons or, as in the present cohort, ultramarathons[514,515]. Furthermore, in this cohort, participants who reported severe

pain intensity on the sf-MPQ and sf-BPI and/ or severe interferences on the sf-BPI also had lower VISA-A scores. In these individuals, pain catastrophising and negative pain perceptions may explain the relationship between pain intensity and its interference with life and well-being as observed in other painful conditions[516–518]. As the link between personality and pain catastrophising has been demonstrated previously[519–522], this strengthens the case for the study of chronic AT pain and personality.

A further finding from this study is that Achilles tendon pain interferes with more than just sporting performance or physical activity. Unsurprisingly, tendon pain interfered with walking as it would likely affect ankle biomechanics during the gait cycle[523]. Tendon pain also interfered with sleep, mood, and enjoyment of life, although mildly. Chronic pain has generally been shown to interfere with these domains in other conditions such as chronic LBP and fibromyalgia[524]. For tendon pain in particular, given the improved sleep[525], mood lifting effects[526,527] and the social aspects[528] that are associated with running and exercise in general, the inability or difficulty in doing so would affect the individuals' enjoyment of life and potentially relationships with others. Consequently, a significant psychological burden as a result of consistent Achilles tendon pain has recently been demonstrated[99,100] and Ceravolo and colleagues[529] have shown that Achilles tendon pain resulted in lowered quality of life in the social and emotional aspects. This effect was even more profound in individuals with a high emotional connection to their physical capabilities. These findings indicate the need to use the biopsychosocial model and to be more holistic in the assessment and hence treatment of Achilles tendon pain.

Modest correlations were observed between complementary aspects of the sf-MPQ, sf-BPI and the VISA-A questionnaires, which implies measurement of similar domains of pain. Similar correlations have been demonstrated in other cohorts when comparing the sf-MPQ and sf-BPI with condition specific pain and interference/disability questionnaires[530]. Individually, each scale measures different aspects of pain[479,487,531] so when taken together, the questionnaires provide a more comprehensive perspective of tendon pain, which can better inform management decisions and also informs future research into the mechanisms of tendon pain[338].

This study is not without limitations. The cohort is from South Africa, a country which has 11 official languages. As a result, although everyone was literate in English, for most, it is not their first or only language, and the accuracy of the sf-MPQ in particular is dependent on one's verbal repertoire[532], which might differ depending on one's ethnicity and gender[487]. Lastly, further studies should include cohorts of athletes whose tendon pain has stopped them from performing load bearing exercise. Understanding the extremes of this phenotype would provide us with more information on the effects of chronic tendon pain on sport participation. The present cohort were an active population who continued to train and participate in their respective sports; a sizeable proportion of the participants reported no present pain on both the sf-MPQ and sf-BPI therefore their pain behaviour may not be entirely representative of the whole cohort of people with Achilles tendinopathy or of those who present for treatment.

In conclusion, tendon pain is largely a continuous type of sensory pain, which may present as different clinical subtypes. Tendon pain interferes with a number of

functional activities including sleep and relations with other people however, this interference is minimal. Lower VISA-A scores were only reported in this cohort when the Achilles tendon pain was generally described as severe or had a moderate to severe impact on daily activities and well-being. This may be as a result of the personalities of the individuals who participate in the kind of sports that are over-represented in the tendinopathy demographics. Lastly, it is noted that using more than the VISA-A to assess tendon pain may be beneficial in providing a more holistic understanding and hence management of tendon pain.

CHAPTER THREE

THE STRUCTURAL CHANGES HYPOTHESIS

A condensed version of this chapter has been published as:

Mkumbuzi, N.S., **Jorgensen, O., Mafu, T.S., September, A.V., Posthumus, M., & Collins, M (2020)**. Tendon structure is not related to self-reported symptoms in recreational runners with chronic Achilles tendinopathy. *Translational Sports Medicine* **3(6):** 589-598; DOI: 10.1002/tsm2.183.

3.1. INTRODUCTION

As described in *Section 1.3* of **Chapter 1**, tendon pain is often notoriously unresponsive to treatment and can persist despite aggressive management strategies such as exercise and corticosteroid injections[106]. This may be due to a poorly understood pathophysiology. Consequently, various hypotheses have been put forward to explain tendon pain and most are based on the abnormal changes that occur in pathologic tendons.

Normal tendons have uniformly arranged fibres, are brilliant white and relatively avascular while abnormal tendons have more bound water, disorganised fibres and have tortuous vessels especially on their anterior surface[257]. Additionally, biomechanical studies have shown that the tendon mechanical properties such as tendon strain are reduced in painful tendons[256]. However, it is generally not possible to have histological samples or access to biomechanical testing in clinical practice. Therefore, imaging is often used to visualise these structural changes in the tendon and confirm diagnoses.

In particular, grey scale US and CD are commonly used as they are relatively ubiquitous, cost effective, allow for dynamic examination and are even mobile[85,246]. However, studies on the relationship between patient reported symptoms and ultrasound findings are equivocal at best. The variability in patients' clinical presentations possibly contributes to the spectrum of observations at ultrasound. This observed spectrum is further confounded by the lack of consensus on what constitutes normal, abnormal or pathological tendon ultrasounds[35,114]. Furthermore, though the literature on tendon structure and clinical presentation is relatively well documented, the relationship between US and CD findings, and tendon pain still remains conflicting.

Some authors have shown that hypoechoic and thickened tendons are painful[257], neovascularisation is associated with pain in some or all tendons[276,277], and that ablation of neovessels led to improvements in pain in athletes with tendon pain[277]. This would suggest that perhaps structural abnormalities may be, at least in part, related to tendon pain and as such ultrasound changes can be used as prognostic markers in treatment[35]. However, positive clinical outcomes and pain resolution have not been associated with changes in tendon structure[12,292,293,300].

On the other hand, some authors have noted absence of neovessels in painful tendons[289] and that some painful tendons are normoechoic[254]. Others have shown that 'normal', pain free tendons also have ultrasound abnormalities such as increased thickness, hypoechogenecity and neovascularisation[13,290,291]. Currently, there are no objective measures for AT severity and improvement. Therefore, more studies are required in the area in order to develop a larger knowledge base from which to make grounded statements on the subject. The lack of consensus in the literature could also

be because previous studies have only used the VISA-A scale as the measure of tendon pain. However, as demonstrated in the previous chapter, using other multidimensional pain scales to characterise Achilles tendon pain provides a more in-depth analysis of the pain. Perhaps, thus far, the conflicting evidence on the relationship between tendon structure and pain could be explained, at least in part, by an insufficient interrogation of tendon pain, which limits any analyses thereafter.

Therefore, the aim of this chapter of the thesis was to assess the association between self-reported pain using three pain questionnaires: VISA-A, sf-MPQ and sf-BPI, with CD and grey scale US findings in recreational athletes with chronic Achilles tendinopathy.

3.2. MATERIALS AND METHODS

3.2.1. Participants

Two hundred and eighty two (282) recreational athletes with a present history of chronic AT (TEN, n=127) together with those without a previous history of AT (CON, n=103) agreed to participate in the study, which was advertised within the greater Cape Town area (South Africa) from November 2017 to July 2018 (Appendix B). As described in the previous chapter, participation in the study was voluntary and all procedures adhered to the Declaration of Helsinki. Ethical clearance and annual review to conduct the study was obtained from the Human Research Ethics Committee of the University of Cape Town's Faculty of Health Sciences (HREC 279/2016-Appendix A). Participants signed an informed consent form prior to participation in the study (Appendix D). Participants completed demographic, medical and pain questionnaires as described in Section 2.2 of **Chapter 2** (Appendix E). Since only recreational athletes with a present history of chronic AT (TEN) where investigated in **Chapter 2**, the inclusion and exclusion criteria of both the TEN and CON participants are presented in Table 3.1.

Table 3.1: Inclusion and exclusion criteria for the participants with a present history of chronic Achilles tendinopathy (TEN) and those without a present or previous history of Achilles tendinopathy (CON).

Inclusion criteria	Exclusion criteria
TEN: - Posterior limb pain in the Achilles tendon for at least 3 months plus any one of: • early morning pain over the mid-portion of the Achilles tendon • early morning stiffness over the mid-portion of the Achilles tendon • a history of swelling over the Achilles tendon area • tenderness to palpation of the mid-portion of the Achilles tendon • palpable nodular thickening over the affected Achilles • movement of the painful area in the Achilles tendon with plantar-dorsi-flexion (positive ''shift'' test)[244] **CON:** - No past or current history of Achilles tendinopathy	**Both TEN and CON:** - Less than one year's experience in main sport, - Younger than 18 years old, - BMI≥30, - Congenital or acquired lower limb deformities, - Surgery in the last 2 years, - Taking chronic medications, - Diagnosed connective tissue disorders - Systemic diseases associated with tendinopathy[42] - History of current or past fluoroquinolone antibiotic use (within the last 12 months) **TEN:** - A history of Achilles tendon rupture, - Insertional tendinopathy, - Achilles tendinopathy of less than 3 months - Local corticosteroids injection in the Achilles tendon or the area surrounding the Achilles tendon prior to the onset of symptoms (≤12 months)

3.2.2. Ultrasound and colour Doppler protocol

Participants were requested to refrain from their regular exercise activity at least 24 hours prior to the US and CD assessment. The ultrasound protocol was performed on all participants after written consent was obtained but prior to clinical confirmation of eligibility criteria. Therefore, the operator was blinded to the clinical conditions of the participants until after the ultrasound protocol.

The participants were examined while lying in prone with fully extended knees and their relaxed, neutral feet overhanging the edge of the examination table. The same clinician (Nonhlanhla Mkumbuzi), with training in musculoskeletal ultrasound, captured 2D mode US images of both Achilles tendons with a 45mm long high-frequency (2-12 MHz) linear array transducer on a Sonoline Antares machine (Siemens Medical Solutions, Erlangen, Germany). The ultrasound acquisition parameters remained constant for all participants and included: transducer centre frequency 8 MHz, depth 2.5cm, and 1 transmit focus point. The examination was performed along the entire length of the tendon in both the sagittal and transverse planes. Particular care was taken to ensure that the probe was aligned with the direction of the tendon fibres. Tendon thickness was measured in millimetres by the maximum anteroposterior diameter in a transverse scan at a neutral position of the ankle. An abnormality at US examination was defined as one or more hypoechoic and/or hyperechoic areas evident in both the longitudinal and transverse scans or an area of thickening with or without hypoechoic areas.

CD was also performed for the entire tendon for all the participants' tendons regardless of grey scale status. The instrument settings for CD examination were optimised for

slow flow (velocity and volume) with a gain of 68 dB, sensitivity of 8 cm/s and a pulse repetition frequency of 1250Hz. For the Doppler images in particular, probe pressure was kept to a minimum to avoid blood vessel occlusion.

Following the US protocol, the participants were then screened for inclusion in the study. If eligible, they would proceed to completing the demographic and pain questionnaires, if not, their US results were not included in the analysis. The analysis of the ultrasound images was conducted by a reviewer (Dr Oscar Jørgensen) who was blinded to the clinical conditions of the participants. Grey scale abnormalities were scored on a dichotomous scale of yes or no pathology present while Doppler results were categorised using the Ohberg scale[275].

3.2.3. Pain Self-report

The participants with Achilles tendinopathy completed the self-administered eight question VISA-A (Appendix F) , the sf-MPQ (Appendix G) and the sf-BPI (Appendix H), as previously described in Section 2.2.2 of the previous chapter.

3.2.4. Statistical analyses

Demographic and clinical data were analysed using Statistica Version 13.2.50. Shapiro-Wilk's test was used to assess the normality of quantitative variables. Normally distributed data were presented as means with standard deviations whereas non-normally distributed quantitative data were presented as medians with interquartile ranges. Categorical data were presented as percentage frequencies. Independent sample t-tests on both normal and log-transformed non-normal data were done to evaluate differences in quantitative demographic characteristics between cases and controls. The Fisher's exact test and Chi-square analyses were used to evaluate differences in categorical demographic, ultrasound scan and colour Doppler characteristics between cases and controls. Comparative analyses for the primary outcomes (pain scores) were performed using t-tests and one-way ANOVA for normally distributed data, and Mann Whitney-U tests and Kruskall-Wallis tests for non-normally distributed data. Wilcoxon's signed rank test was used to evaluate intra-individual differences in tendon diameter between injured and uninjured or dominant and non-dominant limbs. Mardia's multivariate normality test, using the R package "MVN" version 5.8, was used to evaluate bivariate normality for pairs of quantitative variables between tendon diameter and pain scores. Pearson's and Spearman's correlation tests for bivariate normal and bivariate non-normal variable pairs, respectively, were performed to evaluate associations between tendon diameter and pain scores. R package "SpearmanCI" version 1.0 was used to compute 95 % confidence intervals for Spearman's correlations. R package "ggplot2" version 3.2.1 was used to produce all graphs. The level of significance was set as $p<0.05$.

3.3. RESULTS

As illustrated in Figure 3.1, of the 282 participants who were recruited for the study, 103 controls (CON) without a previous history of AT and 127 cases (TEN) with a present history of chronic AT were included.

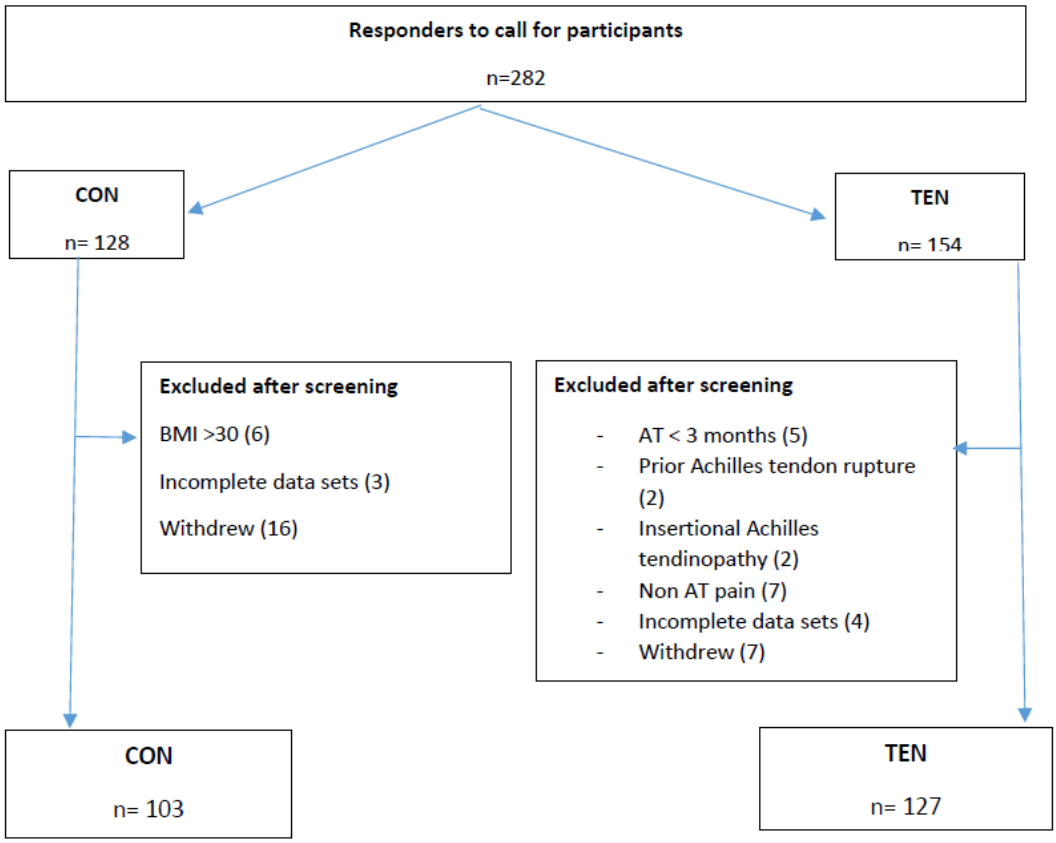

Figure 3.1: Flow of participants with a present history of chronic Achilles tendinopathy (TEN) and those without a previous history of Achilles tendinopathy (CON) included in the study (AT- Achilles tendinopathy; BMI- body mass index). The numbers of participants for whom each criterion applied are presented in parentheses.

In some instances, some specific data are missing hence, the numbers in some of the participants in the tables do not add up to the total number of participants. There were

no significant differences in sex, height, weight, dominant leg, number of years of participation in sport and hours of training per week during the previous two years between athletes with AT and the controls (Table 3.2). Participants with tendinopathy reported symptoms and median VISA- A scores for an average [median (IQR)] of 12 months (6.0; 36.0) and 72 (61 – 82), respectively. They were also significantly older, with higher BMIs and were predominantly runners (Table 3.2).

With regards to affected side, 67% (n=82) had unilateral tendinopathy while 33% (n=41) were affected bilaterally. Comparisons showed that those with bilateral tendinopathy reported symptoms for longer (months) [median (IQR)] [20 (8.0- 45.0)], than those with unilateral symptoms [12.0 (5.0 - 24.0)], p=0.009. No other significant differences were noted between those affected unilaterally and those affected bilaterally (Table 3.3).

In unilateral tendinopathy, 68.1% (n=56) were affected on their non-dominant leg. The median (IQR) of the injured tendons were significantly thicker [6.3mm (5.4 - 7.9)] than the uninjured tendons [5.7mm (5.0 - 7.1)] ($p<0.001$) (Table 3.4). The injured tendon also had abnormal ultrasound appearance (49% vs 24%) (p=0.003) and tended towards more neovessels (20.5% vs 9.0%) (p=0.069) when compared to their uninjured tendons. None of these differences were attributable to leg dominance within the TEN groups, since the diameters, ultrasound appearance and neovascularisation between the dominant and non-dominant legs of the controls, as well as between the uninjured limbs of the TEN and CON groups, were similar ($p>0.05$) (Table 3.4).

Table 3.2: Demographic characteristics of healthy controls without a history of Achilles tendinopathy (CON) and participants with a present history of chronic Achilles tendinopathy (TEN).

	CON (n=103)	TEN (n=127)	p-value
Age at recruitment (years)	37.0 (31.0 - 50.0)	46.0 (36.0 - 52.0)	**0.004**
Sex (% male)	62.9 (66)	61.2 (79)	0.892
Height (cm)	173.6 ± 8.9	172.9 ± 9.2	0.568
Mass (kg)	70.8 (60.0 - 80.0)	75.0 (66.5 - 85.0)	0.053[a]
BMI ($kg.m^{-2}$)	23.3 (20.7 - 25.4)	24.8 (22.9 - 27.8)	**0.001[a]**
Dominant Leg (% right)	87.4 (90)	77.5 (93)	0.149
Main Sport (% Running)	62.4 (63)	80.5 (95)	**0.004**
Training level (hours/week)			
Past 3 months	5.0 (3.0 - 7.0)	5.0 (3.0 - 7.0)	0.850
Past 12 months	5.0 (3.0 - 7.0)	6.0 (4.0 - 8.0)	0.230
Past 24 months	5.0 (3.0 - 6.0)	6.0 (3.0 - 8.0)	0.269
Duration of participation in main sport (years)	10.0 (5.0 - 19.0)	9.0 (4.5 - 20.0)	0.597

Height is expressed as mean ± standard deviation, while sex, dominant limb and main sport are represented as a percentage with n in parentheses. The remaining variables are expressed as median (IQR).
[a] adjusted for age
Ambidextrous: 3.9% (n=4) CON and 8.3% (n=10) TEN
CON non-running main sports: 3.0% (n=3) cricket/golf; 11.9% (n=12) cycling, 3.0% (n=3) gym; 5.0% (n=5) hiking and 14.9% (n=15) other. TEN non-running main sports: 3.4% (n=4) cricket/golf; 1.7% (n=2) cycling, 4.2% (n=5) gym; 2.5% (n=3) triathlon and 7.6% (n=9) other.

Table 3.3: Comparisons between participants with a present history of unilateral and bilateral chronic Achilles tendinopathy (AT).

	Unilateral AT (n=82)	Bilateral AT (n=41)	p-value
Age (years)	44.8 ± 11.7	43.4 ± 8.3	0.486
Sex (% male)	65.9 (54)	53.7 (22)	0.238
Height (cm)	173.8 ± 8.8	171.6 ± 9.2	0.207
Mass (kg)	75.4 ± 13.2	74.2 ± 14.4	0.654
BMI ($kg.m^{-2}$)	24.8 (22.6 - 28.0)	24.8 (23.1 - 27.6)	0.800
Dominant Leg (% right)	72.2 (57)	89.2 (33)	0.115
Main Sport (% Running)	82.1 (64)	80.6 (29)	0.802
Training level (hours/week)			
Past 3 months	5.0 (3.0 - 7.0)	6.0 (3.0 - 7.0)	0.992
Past 12 months	6.0 (4.0 - 8.0)	6.0 (4.0 - 8.0)	0.556
Past 24 months	5.5 (3.0 - 8.0)	6.0 (2.0 - 8.0)	0.634
Duration of participation in main sport (years)	10.0 (5.0 - 20.0)	9.0 (4.0 - 20.0)	0.803
Duration of symptoms (months)	12.0 (5.0 - 24.0)	20 (8.0 - 45.0)	**0.009**

Height is expressed as mean ± standard deviation, while sex, dominant limb and main sport are represented as a percentage with n in parentheses. The remaining variables are expressed as median (IQR).
Ambidextrous: 10.1% (n=8) unilateral AT and 2.7% (n=1) bilateral AT
Unilateral AT non-running main sports: 1.3% (n=1) cricket/golf; 1.3% (n=1) cycling, 3.9% (n=3) gym; 2.6% (n=2) triathlon and 9.0% (n=7) other.
Bilateral AT non-running main sports: 5.6% (n=2) cricket/golf; 2.8% (n=1) cycling, 2.8% (n=1) gym; 2.8% (n=1) triathlon and 5.6% (n=2) other.
Only participants who had full data sets were included for analysis in this table, 4 participants did not complete all the information required.

Comparisons between the dominant and non-dominant legs in the TEN participants with bilateral AT showed they had similar diameters (p=0.955), ultrasound appearance (p=1.000) and neovascularisation (p=0.195) (Table 3.5). For these reasons (i) the injured leg in those with unilateral tendinopathy and (ii) the dominant leg in those with

bilateral symptoms was analysed in the TEN group while the dominant leg was analysed in the CON participants.

Table 3.4: Comparisons of tendon diameter, echogenicity (presence of pathology/ultrasound abnormalities) and neovascularisation (Ohberg score) between the injured and uninjured Achilles tendons in participants with a present history of unilateral chronic Achilles tendinopathy (Unilateral TEN), as well as the dominant and non-dominant tendons in healthy controls (CON) without a previous history of Achilles tendinopathy. The total number of participants (n) in each group are given.

		CON			**Unilateral TEN**		
		Dominant (n=86)	**Non-dominant (n=84)**	**p-value**	**Injured (n=78)**	**Uninjured (n=78)**	**p-value**
Tendon diameter (mm)		5.5 (4.8 - 6.0)	5.7 (4.9 - 6.1)	0.939	6.3 (5.4 - 7.9)	5.7 (5.0 - 7.1)	**<0.001**
Ultrasound abnormalities (% yes)		22.4 (19)	20.2 (17)	0.851	48.7 (38)	24.4 (19)	**0.003**
Ohberg score: (% yes)	**0**	99.0 (85)	99.0 (83)		79.5 (62)	91.0 (71)	
(% yes)	**1**	1.0 (1)*	1.0 (1)*	1.000	15.4 (12)	3.9 (3)	0.069
(% yes)	**2**	0.0 (0)	0.0 (0)		5.1 (4)	5.1 (4)	

Tendon diameter is expressed as expressed as median (IQR) and presence of pathology and Ohberg scores are represented as a percentage with n in parentheses.
* This was the same individual.

When comparing the injured Achilles tendon in the TEN group with the dominant tendon of the CON group, the TEN participants had thicker tendons (median [IQR]) [TEN 6.3 mm (5.4 - 7.9) vs CON 5.5 (4.8 - 6.0), p<0,001]; relative abnormal ultrasound appearances (TEN 48.7%, n=38 vs CON 22.4%, n=19, p=0.001) and had more

neovessels (TEN 20.5%, n=16 vs CON 1.0%, n=1, p=0.001) (p<0.001) than the CON participants (Table 3.4).

Table 3.5: Comparisons of tendon diameter, echogenicity (presence of pathology/ultrasound abnormalities) and neovascularisation (Ohberg score) between the dominant and non-dominant legs of participants with bilateral Achilles tendinopathy.

		Dominant leg (n=35)	Non-dominant leg (n=35)	p-value
Tendon diameter (mm)		5.8 (5.1 - 6.8)	5.8 (4.9 - 8.0)	0.955
Ultrasound abnormalities (% yes)		42.9 (15)	45.7 (16)	1.000
Ohberg score:	**0** (% yes)	84.2 (30)	79.4 (27)	
	1 (% yes)	2.9 (1)	14.7 (5)	0.195
	≥2 (% yes)	8.8 (3)	5.9 (2)	

Tendon diameter is expressed as expressed as median (IQR) and presence of pathology and Ohberg score are represented as a percentage with n in parentheses.

Except for the VISA-A interference index scores and tendon US appearance, no significant differences were noted between the self-reported total pain scores of the sf-MPQ, sf-BPI and VISA-A scales or their separate indices (sensory, interreference, affective, intensity) and tendon diameter, US abnormalities or presence of neovessels (p>0.05) (Tables 3.6 to 3.8). The median interference index scores of the VISA-A questionnaire of participants with the presence of US abnormalities [35.5 (30.0-41.0), n=36] was significantly higher than those with the absence of abnormalities [32.5 (26.0-37.0), n=39, p=0.046] (Table 3.7).

Table 3.6: Correlations between tendon diameter of the injured tendons and pain scales indices from the Victorian Institute of Sports Assessment – Achilles questionnaire (VISA-A), short form of the McGill pain questionnaire (sf-MPQ) and short form of the Brief Pain Inventory (sf-BPI) in participants with chronic Achilles tendinopathy (TEN).

	r (n=65) [a]	95 % CI	p-value
VISA-A			
Total score	0.102	-0.151 to 0.356	0.421
Sensory index	0.048	-0.214 to 0.310	0.708
Interference index	0.085	-0.158 to 0.327	0.505
sf-MPQ			
Total score	0.077	-0.202 to 0.356	0.543
Sensory index	0.047	-0.311 to 0.216	0.707
Affective index	0.002	-0.271 to 0.275	0.986
sf-BPI			
Total score	-0.153	-0.422 to 0.115	0.226
Intensity index	0.011	-0.255 to 0.276	0.933
Interference index	-0.206	-0.455 to 0.043	0.103

Except for the total VISA-A scores which are expressed as means ± standard deviation, the rest of the variables are presented as medians (IQR).
[a] Pearson's correlation was used to analyse the correlation between the VISA-A scores and the tendon diameters. The rest of the variables were assessed using Spearman's correlation
Only 65 participants had a full data set: completed, returned all 3 pain questionnaires and tendon diameter data.

Table 3.7: Associations between grey scale ultrasound (US) observations of the injured tendons and pain scales indices from the Victorian Institute of Sports Assessment – Achilles questionnaire (VISA-A), short form of the McGill pain questionnaire (sf-MPQ) and short form of the Brief Pain Inventory (sf-BPI) in participants with chronic Achilles tendinopathy (TEN).

Pathology on greyscale US	Absent (n = 39)	Present (n = 36)	p-value
VISA-A			
Total score	67.8 ± 14.6	75.1 ± 16.2	0.078
Sensory index	39.0 (35.0 - 45.0)	41.5 (35.5 - 48.0)	0.184
Interference index	32.5 (26.0 - 37.0)	35.5 (30.0 - 41.0)	**0.046**
sf-MPQ			
Total score	7.0 (4.0 - 9.1)	7.7 (4.1 - 12.0)	0.420
Sensory index	3.0 (2.0 - 6.0)	3.0 (2.0 - 7.5)	0.966
Affective index	0.0 (0.0 - 1.0)	0.0 (0.0 - 1.0)	0.984
sf-BPI			
Total score	18.0 (8.0 - 27.0)	12.5 (7.0 - 24.5)	0.267
Intensity index	11.0 (6.0 - 14.0)	8.5 (6.0 - 12.0)	0.343
Interference index	9.5 (2.0 - 14.0)	4.0 (1.0 - 12.0)	0.285

Except for the total VISA-A scores which are expressed as means ± standard deviation, the rest of the variables are presented as medians (IQR).
Only 75 participants had a full data set: completed and returned 3 pain questionnaires and had readable ultrasound scans.

Table 3.8: Associations between neovascularisation, as measured by the Ohberg Score, of the injured tendons and pain scales indices from the Victorian Institute of Sports Assessment – Achilles questionnaire (VISA-A), short form of the McGill pain questionnaire (sf-MPQ) and short form of the Brief Pain Inventory (sf-BPI) in participants with chronic Achilles tendinopathy (TEN).

	0 (n = 60)	1 (n = 11)	2 (n = 4)	p-value
VISA-A				
Total score	71.1 ± 16.4	76.5 ± 13.2	69.8 ± 9.1	0.557
Sensory index	39.0 (33.0 - 46.0)	42.0 (38.0 - 48.0)	40.5 (33.5 - 47.5)	0.337
Interference index	34.0 (26.0 - 40.0)	34.0 (27.0 - 40.0)	32.0(27.5 - 34.5)	0.746
sf-MPQ				
Total score	6.6 (3.0 - 9.8)	11.0 (6.4 - 13.2)	6.7 (4.2 - 15.3)	0.149
Sensory index	3.0 (2.0 - 6.0)	4.0 (2.0 - 7.0)	4.5 (2.0 - 10.0)	0.551
Affective index	0.0 (0.0 - 1.0)	0.0 (0.0 - 1.0)	0.0 (0.0 - 2.5)	0.974
sf-BPI				
Total score	16.0 (7.0 - 29.0)	14.0 (8.0 - 21.0)	11.5 (11.0 - 14.5)	0.615
Intensity index	10.0 (6.0 - 14.0)	9.0 (7.0 - 12.0)	8.0 (6.5 - 10.5)	0.919
Interference index	8.0 (2.0 - 17.0)	2.0 (1.0 - 11.0)	3.0 (1.0 - 7.5)	0.285

Except for the total VISA-A scores which are expressed as means ± standard deviation, the rest of the variables are presented as medians (IQR).
Only 75 participants had a full data set: completed and returned 3 pain questionnaires and had readable CD scans.

When compared to participants who reported no stabbing pain, those who reported mild, moderate or severe stabbing pain on the sf-MPQ had significantly thicker tendons, [median (IQR)] [6.0mm (5.2 – 7.6) vs 7.0mm (5.9 – 8.9), 7.7mm (6.2 – 9.1) and 6.3mm (4.9 – 7.4)] (p=0.037) (Table 3.9). When considering the other common descriptors used by the participants to describe their pain **(Chapter 2)**, the thickness of the affected tendon thickness was however similar between those who reported no

pain and the severity of pain for all the other descriptors or combinations of descriptors (Table 3.9). Similarly, no significant correlations were observed between tendon diameter and the VAS score of the sf-MPQ (Spearman's r=-0.084, p=0.404, n=101).

Table 3.9: The relationship between the median (IQR) diameter (mm) of the affected Achilles tendon and self-reported pain severity (none, mild, moderate and severe) for the seven most commonly used pain descriptors within the sensory index of the short form McGill Pain Questionnaire (sf-MPQ).

	None	Mild	Moderate	Severe	p- value
Aching	6.1 (5.1- 8.2), 36	6.2 (5.3-8.0), 35	5.8 (5.2-7.5), 25	6.7 (5.7-9.6), 5	0.928
Tender	6.0 (5.0-7.3), 52	6.9 (5.4-8.1), 22	6.8 (5.3-9.3), 18	6.2 (4.9-7.2), 9	0.059
Throbbing	6.2 (5.3-7.9), 67	6.6 (5.4-8.6), 19	5.7 (5.1-6.4), 6	6.3 (5.1-8.7), 9	0.936
Sharp	6.2 (5.3-7.8), 68	5.7 (5.3-6.7), 15	7.4 (5.4-9.8), 10	5.8 (4.9-7.9), 8	0.834
Stabbing	6.0 (5.2-7.6), 73	7.0 (5.9-8.9), 19	7.7 (6.2-9.1),9	6.3 (4.9-7.4), 6	**0.037**
Shooting	6.2 (5.3-7.9), 77	5.6 (5.3-6.8), 8	7.1 (5.8-8.8),11	6.6 (4.6-9.0), 5	0.617
Hot burning	6.2 (5.3-7.6), 75	5.9 (5.3-8.1), 13	8.1 (6.2-10.0), 9	5.7 (4.9- 6.5), 4	0.516
Continuous [a]	5.9 (4.9-7.4), 14	6.2 (5.4-8.0), 39	6.1 (5.2-8.1), 34	6.3 (5.2-7.8), 14	0.280
Intermittent [b]	6.1 (5.1-7.6), 51	5.9 (5.4-7.4), 19	6.9 (5.7-8.9), 18	6.3 (5.0-8.1), 13	0.238
All [c]	6.2 (5.3-7.8), 41	5.9 (5.4-7.2), 25	7.0 (5.6-9.0), 21	6.3 (5.0-8.0), 14	0.418

The number of participants (n) in each group is indicated after the median (IQR) values; p-values (none vs mild, moderate plus severe).
[a] Aching, Tender and Throbbing; [b] Sharp, Stabbing and Shooting; [c] all seven descriptors
Participants selected none for all of the individual descriptors to be included in the none group of the continuous, intermittent or all groups. For the mild, moderate and severe groups, at least one of the descriptors was described with a maximum pain of mild, moderate or severe, respectively.
Only 101 participants completed and returned the sf-MPQ pain questionnaires and had readable US scans.

In contrast, when considering the common pain descriptors reported by the participants on the PPI scale of the sf-MPQ **(Chapter 2)** those who reported mild present pain had thicker tendons [7.4 mm (5.9-7.4), n=26] than those with no pain [5.8 mm (5.3-7.9), n=33, p=0.037]. However, there were no differences between tendon

diameters between participants who reported no pain compared to discomforting pain [6.2 mm (5.2-7.9), n=36, p=0.711. There were no significant differences (p=0.610) in the relative number of individuals with US abnormalities within the no (17 out of 42, 40.5%), mild (17 out of 28, 60.7%) and discomforting (18 out of 40, 45.0%) pain groups on the PPI scale. Similarly, there were no significant differences (p=0.857) in the median VAS scores between the groups with absence [0.7 (0.0-3.5), n=63] and presence [0.3 (0.0-3.5), n=54] of US abnormalities.

No significant associations were observed between the diameter of the affected tendon and the extent to which pain interfered with various activities reported on the pain interference domain of the sf-BPI scale. However, participants who reported moderate or severe pain at its worst when compared to those with mild or no pain at its worst had significant thicker tendons (p=0.006) (Table 3.10). There was no other significant difference in tendon diameters between any of the severity groups of the other pain intensity domain descriptors (Table 3.10).

Additionally, there were no associations between the intensity of the seven most commonly chosen sf-MPQ word descriptors and US appearance (Table 3.11). However, presence of ultrasound abnormalities was significantly associated with mild or severe interference with general activity (p=0.020) and walking ability (p=0.038) (Table 3.12). There were no other significant differences between tendon US abnormalities between the severity groups of the other activities reported on the sf-BPI pain interference domain or any of the four pain intensity domain descriptors (Table 3.12).

Table 3.10: The relationship between the median (IQR) diameter (mm) of the affected Achilles tendon and the level of self-reported interference severity (none, mild, moderate and severe) of tendon pain for the domains of the short form Brief Pain Inventory (sf-BPI).

	None	Mild	Moderate & Severe	p-value
Pain interference domain				
Relationships (mm)	6.1 (5.3-7.9), 75	6.6 (5.2-7.9), 20	6.3 (4.9-8.6), 5	0.904
Sleep (mm)	6.3 (5.4-8.1), 67	5.9 (5.3-7.6), 28	5.2 (4.7-5.7), 5	0.097
Mood (mm)	6.2 (5.4-8.0), 52	6.0 (5.2-7.5)	5.4 (5.0-8.0), 17	0.174
General activity (mm)	6.2 (5.4-8.2), 47	5.8 (5.3-7.5), 43	6.5 (5.0-8.2), 10	0.242
Enjoyment of life (mm)	6.2 (5.4-7.8), 49	6.5 (5.2-8.1), 36	5.4 (5.0-7.9), 15	0.603
Normal work (mm)	6.2 (5.4-8.0), 46	6.0 (5.3-7.8), 45	5.4 (4.9-8.0), 9	0.395
Walking ability (mm)	6.2 (5.2-7.8), 28	6.0 (5.3-8.1), 55	6.7 (5.2-8.2), 17	0.904
Pain intensity domain				
At present (mm)	6.0 (5.4-7.9), 35	6.3 (5.3-8.2), 57	5.1 (4.6-6.5), 8	0.069
On average (mm)	7.5 (5.3-8.0), 3	6.2 (5.4-8.0), 73	5.8 (4.9-7.6), 24	0.139 [a]
At its least (mm)	6.2 (5.3-7.7), 36	6.2 (6.4-8.0), 54	5.5 (4.7-8.1), 10	0.826
At its worst (mm)	5.9 (4.7-10.3), 3	6.7 (5.8-8.2), 51	5.4 (5.1-7.0), 46	**0.006** [a]

The number of participants (n) in each group is indicated after the median (IQR) values; p-values (none vs mild, moderate plus severe).
[a] None plus mild vs moderate plus severe
Only 100 participants completed and returned the sf-BPI pain questionnaires and had readable US scans.

Table 3.11: The relationship between presence of ultrasound abnormalities in the affected Achilles tendon and self-reported pain severity (none, mild, moderate and severe) for the seven most commonly used pain descriptors within the sensory index as well as for the grouped pain descriptors (continuous and intermittent pain) of the short form McGill Pain Questionnaire (sf-MPQ).

	None	Mild to Severe	p-value
Aching (% Yes)	50.0 (46)	45.2 (71)	0.707
Tender (% Yes)	39.3 (56)	54.1 (61)	0.139
Throbbing (% Yes)	44.9 (78)	50.0 (40)	0.697
Sharp (% Yes)	42.3 (78)	56.4 (39)	0.172
Stabbing (% Yes)	43.6 (85)	56.3 (32)	0.299
Shooting (% Yes)	44.3 (88)	55.2 (29)	0.392
Hot burning (% Yes)	46.6 (88)	48.3 (29)	1.000
Continuous pain (% Yes) [a]	41.2 (17)	48.0 (100)	0.794
Intermittent pain (% Yes) [b]	40.4 (57)	53.3 (60)	0.196
All (% Yes) [c]	44.4 (9)	47.2 (108) [d]	1.000

[a] Aching, Tender and Throbbing; [b] Sharp, Stabbing and Shooting; [c] all seven descriptors
[d] Mild 35.9% (n=39), Moderate 55.6% (n=45) and Severe 50.0% (n=24)

Table 3.12: The relationship between ultrasound abnormalities in the affected Achilles tendon and the level of self-reported interference severity (none, mild, moderate and severe) of tendon pain for the domains of the short form Brief Pain Inventory (sf-BPI).

	None	Mild	Moderate to Severe	p-value
Pain interference domain				
Relationships (% Yes)	47.1 (87)	39.1 (23)	66.7 (6)	1.000 [a]
Sleep (% Yes)	48.7 (78)	45.5 (33)	20.0 (5)	0.555 [a]
Mood (% Yes)	42.1 (57)	45.0 (40)	63.2 (19)	0.273
General activity (% Yes)	50.9 (53)	35.3 (51)	76.9 (13)	**0.020**
Enjoyment of life (% Yes)	36.5 (52)	53.2 (47)	58.8 (17)	0.138
Normal work (% Yes)	47.1 (51)	43.6 (55)	60.0 (10)	1.000 [a]
Walking ability (% Yes)	33.3 (30)	45.5 (66)	70.0 (20)	**0.038**
Pain intensity domain				
At present (% Yes)	42.5 (40)	52.3 (65)	27.3 (11)	0.562 [a]
On average (% Yes)	50.0 (4)	47.6 (84)	42.9 (28)	0.671 [b]
At its least (% Yes)	48.9 (43)	46.0 (63)	40.0 (10)	0.847 [a]
At its worst (% Yes)	50.0 (4)	51.7 (60)	40.4 (52)	0.264 [b]

[a] None vs mild to severe
[b] None and mild vs moderate and severe

3.4. DISCUSSION

The main findings of this chapter of the thesis were that, recreational athletes with chronic AT presented with (i) abnormal tendon US findings when compared to pain free controls; (ii) thicker tendons, (iii) presence of neovessels and (iv) altered echogenicity in the involved tendons. Additionally, in this study, (i) mild to severe stabbing pain, (ii) mild, but not discomforting, present pain, as well as (iii) moderate to severe pain at its worst were associated with thicker tendons. Interference index scores of the VISA-A questionnaire were also higher in participants with US abnormalities. US abnormalities were also associated with interference with general activity and walking ability in this study. Although a number of researchers have previously investigated the association between VISA-A scores with tendon structural changes, this is the first study to investigate the possible association of Achilles tendon pain as reported on additional pain scales, namely the sf-MPQ and sf-BPI questionnaires, with abnormal US findings.

Participants with tendinopathy presented with abnormal tendons on US when compared to asymptomatic controls. These data are consistent with previous studies that have shown that pathological tendons tend to have alterations in echogenicity as a result of the pathology[306,533,534]. A study by Palaniswamy et al.[279] showed that compared to asymptomatic controls, individuals with tendinopathy presented with structural changes on ultrasound. These changes are likely an indication of the changes occurring in the tendon itself. Tendinopathy leads to the loss of the parallel fibrillar arrangement, retention of more bound water and hence changes in sonographic appearance[257]. However, as did Palaniswamy et al.[279], it was noted in

this study that a substantial proportion of asymptomatic tendons also had abnormal US findings. Moreover, some symptomatic tendons in this cohort were normoechoic.

While the relationship between presenting tendinopathy and US findings may not be unequivocal, abnormal imaging findings may hold some prognostic value. Studies have consistently shown that US abnormalities at baseline in asymptomatic tendons predict poor outcomes from six months[35,258] and tendons that are homogenous on US but painful are associated with better response to treatment compared to heterogeneous, painful tendons though not always[300]. These collective observations highlight the need to further decipher the clinical utility of tendon structure, patient presentation and US findings in tendinopathy. Such findings are all the more important for those athletes who do not necessarily experience pain as a warning signal. US imaging could be used to track their tendinopathy risk through the season and institute preventive measures, as necessary.

Another finding in the present study was that there was minimal neovascularisation in this cohort. Where present, there was a trend towards it being significantly overrepresented in the symptomatic tendons, however, the small sample sizes may have been a limitation in further elucidating this phenomenon. This trend mirrors previous literature, which has noted neovascularisation as a feature of symptomatic tendinopathy[276]. The importance of neovessels in tendinopathy is underscored by the close anatomical interaction between nerve fibres and blood vessels in pathological tendons[106]. This has led to the neovascularisation hypothesis as a potential explanation for tendon pain. Moreover, previous studies have shown that the presence of neovessels results in an increased risk of developing AT with some authors showing

this risk to be as high as sevenfold[280]. However, these findings have not been reported consistently[254]. Additionally, neovessels are not a constant finding as even in this cohort, they were not observed in over 80% of the participants. This value is within the rather wide ranges established in previous studies that have observed neovessels in as little as 4% in some cohorts and up to 97% in others[305]. The discrepancies in these findings could be because neovascularisation is a dynamic finding, which could be pronounced during acute exacerbations of pain or bouts of activity then abate by the time of testing[535]. In this study, participants were requested to refrain from physical activity 24 hours prior to the testing procedure to prevent this.

The general disconnect between US and/or CD findings and tendon pain is consistent with results from previous studies, which demonstrated a similar relationship, or lack thereof, in spite of observing neovascularisation in symptomatic tendons[158,287,296,299,305,536]. Neovascularisation and VISA-A scores were also independent in a study by Cheng et al.[537] who observed improvements in VISA-A scores with shock wave therapy irrespective of neovascular status. De Marchi et al.[295] also showed no relationship even though they used CEUS, which is considered more sensitive than CD at detecting blood vessels. In contrast, various other authors have shown that tendon pain is associated with presence of[276,305], volume and position of neovessels[112] tendon size and abnormalities in the tendon matrix[257,279]. Additionally, some authors have shown that ablation of these neovessels leads to improved pain report[538]. On the other hand, Durcan et al.[539] showed that the more normoechoic the patellar tendons were in academy rugby players, the higher their VISA- P scores were. This non-linear association and contradictory relationship between imaging findings

and the clinical presentation is not unique to tendinopathy though; it has also been observed in other heterogeneous musculoskeletal disorders such as low back pain[540].

Thicker tendons were associated with mild to severe stabbing pain as well as mild but not discomforting pain on the present pain intensity score. It is likely that participants who presented with present mild pain, which includes those whose initial moderate to severe pain was presently mild, would likely train **(Chapter 2)** while those with discomforting or more severe pain would not. Additionally, stabbing pain is an intermittent pain, which is not present continually. As such, perhaps individuals with intermittent pain are more likely to train on these tendons than would those with pain that is continually present. Continued loading to the tendon during mild and/or stabbing pain might delay tendon healing. Tendons in these individuals could therefore remain in the reactive phase of the continuum model of the pathogenesis of tendinopathy[507,541,542]. Therefore, future studies should quantify the tendon loading in order to draw more definitive conclusions on the relationship between tendon loading, imaging findings and patient reported symptoms.

While US abnormalities were not associated with pain in this cohort, they were associated with higher interference index scores of the VISA-A as well as interference scores for general activity and walking ability on the sf-BPI. These findings suggest that impairments in locomotion and function exist in the pathological tendon even in the absence of pain. Hence, using pain as the main outcome following treatment may be insufficient. This assertion is supported by studies that have reported functional deficits after pain resolution with treatment[293] and reiterated by a systematic review that could not find evidence showing that structural changes are responsible for the

response to exercise in tendinopathy rehabilitation[543]. Additionally, positive clinical outcomes have been reported in the absence of normalising of tendon structure[257,305]. Changes in Achilles tendon structure have also been observed in ballet dancers who reported unchanged pain scores[544], which may suggest that structural changes are not associated with pain in tendinopathy but may be associated with function.

Perhaps the technical weaknesses of US and CD could explain these inconsistent observations in the literature. Currently, there is no gold standard objective method to assess tendon pain or to monitor the progress of tendinopathy either during a sporting season or as a part of treatment. Assuming a relationship between structure and function, a good objective measure would correlate the structural changes that occur in tendinopathy with the symptoms reported by the sufferer. US, and in particular CD, is operator dependent, which would lead to great variability in what is considered pathological or normal[305]. Some authors have recently suggested standardised analytical methods in the interpretation of tendon US images[545]. However, more work needs to be done to ascertain the reproducibility of such methods. There is also great variability in machine settings across the various studies, which makes direct comparisons rather challenging. Other imaging modalities such as MRI could be used instead of US and CD; MRI has been shown to be accurate in the diagnosis of tendinopathy[13,245]. However, MRI is more expensive and not readily available to practitioners at the point of care. Together, another drawback of both US and MRI is that one is unable to detect early degeneration or reproducibly monitor progress using either modality. For this, a new modality, UTC, has been developed[546].

Ultrasound tissue characterisation is an innovative modality available to assess tendons. It can be used for 3D rendering of the tendon, tissue characterisation and quantification of tissue architecture[547]. In can discriminate different collagen echo types, which correspond to the histopathological staging of tendinopathy. Studies using UTC have shown that it has a high discriminative power, has high inter- and intra-observer reproducibility[546], is suitable for detection of subtle in-situ tendon responses to load[548,549], able to provide early diagnosis of injury or degeneration[544] and an objective evaluation of treatment effects[536]. One of the strengths of UTC as an imaging modality is that it is more objective compared to US in particular[546] where subjectivity can affect the determination of pathology. With further development of such modalities, it will become easier to investigate the involvement of the tendon itself as a source of pain. A major drawback to the use of UTC is its rather prohibitive cost, which makes it not readily available and the as yet largely unverified assertions of very histological level resolution. Realistically, perhaps it is worth the effort to standardise and refine current CD and US protocols for easier comparisons across studies. In particular because US offers a number of advantages to the clinician such as being easily accessible, not time consuming, the training in its use and interpretation is relatively easy compared to MRI, it is affordable and allows for quick comparison of limbs[85,114] Additionally, to better understand the complex interaction between tendon pain, structure, and function, researchers should start moving towards *in vivo* assessment of the Achilles tendon structure during loading with simultaneous ultrasound recording. Similarly, scientists should also consider administering the pain questionnaires during loading in order to better understand the dynamic relationship between pain, structure and function.

This study is not without limitations. Firstly, it is cross sectional in nature, therefore causation cannot be determined; longitudinal cohort studies with long follow up periods are warranted. Secondly, this study used a binary yes/no scale to determine presence or absence of pathology on US, which leads to a loss of information with grouping. Which, regrettably, does not capture the spectrum of potential structural changes. It is therefore recommended to use scales/grading systems that further delineate tendon structure to assist in characterising the extent of pathology. Additionally, the author's limited experience with sonography could have an effect on the reliability and accuracy of the imaging. However, the investigator surmises this is representative of the average clinician to whom an athlete would present for treatment.

In conclusion, pathological tendons appear abnormal on grey scale US and present with neovessels when compared to normal tendons. However, based on the sf-MPQ, VISA-A and sf-BPI scores, no associations between self-reported pain, US and CD findings in the tendinopathy group were noted. These results add to the available conflicting information on the subject, which could be resolved by standardisation of US protocols or development of more sensitive tests.

CHAPTER 4

CENTRAL PAIN HYPOTHESIS

A condensed version of this chapter has been published as:

Mkumbuzi, N.S., Mafu, T.S., September, A.V., Posthumus, M., & Collins, M. Conditioned pain modulation is not altered in recreational athletes with chronic Achilles tendinopathy. *Translational Sports Medicine* 00: 1-7. DOI: org/10.1002/tsm2.201

4.1. INTRODUCTION

As discussed in earlier chapters, the causes and mechanisms of pain in AT are not yet fully understood. Traditionally, tendinopathy has for the most part been considered as a peripheral condition whose pathology (and hence treatment) is confined to itself and its immediate relations[106,550,551]. However, there are limitations to pain models that assume a peripheral, tissue-based source of tendon pain. Notably, as demonstrated in **Chapter 3**, there is a mismatch between tendon pain and pathology and improvements in clinical outcomes are not necessarily associated with structural restoration[292]. As such, contemporary data suggests that there are probably some CNS drivers for tendon pain acting in isolation or in concert with local pain mechanisms[39,40,552].

In studies of other chronic pain conditions, the CNS has been shown to play a significant role. In particular, an imbalance between central facilitatory and inhibitory pain mechanisms has been identified as a potential cause of chronic pain[309,327]. An overactive facilitatory mechanism would amplify the nociceptive input while impaired endogenous inhibitory mechanisms would not attenuate the nociceptive signal efficiently[309,327], which would lead to dysfunctional endogenous analgesic control. The

endogenous inhibition can be assessed by testing the CPM, which occurs when the response to a painful test stimulus is inhibited by an additional conditioning painful stimulus[315].

Aberrations in the function of the descending pain modulating system as demonstrated by altered CPM have been observed in chronic pain conditions such IBS[330], non-specific LBP[320], CTS[335], patellofemoral join pain[553] and FM[331]. Altered CPM has also been shown in tendinopathies such as LET[133], patellar[340] and shoulder tendinopathies[334]. This may suggest that CPM integrity could be a factor in the development and maintenance of chronic pain conditions. However, the evidence for altered CPM and central pain mechanisms in AT is conflicting. Some authors report a reduced CPM in AT[39], which suggests a central contribution to its pain while others report no central sensitisation when compared to asymptomatic controls[132], which suggests a local source of tendon pain. Given the mixed evidence available and the relative dearth of CPM studies in AT, more work is necessary to interrogate the role of central pain mechanisms in AT, preferably in larger cohorts as recommended in previous work[40,132]. Therefore, the research questions for this chapter were, what is the relationship between AT and CPM in recreational athletes? Additionally, what is the relationship between the CPM and self-reported tendon pain scores?

4.2. MATERIALS AND METHODS

4.2.1. Participants

Participants were recruited and screened for inclusion and exclusion criteria as described in *Section 3.2.1* of **Chapter 3.**

4.2.2. Pain self-report

The participants completed demographic, sporting and medical history questionnaires (Appendix E). They also completed the self-administered eight question VISA-A (Appendix F), the sf-MPQ (Appendix G) and the sf-BPI (Appendix H) as described in *Section 2.2.2* of **Chapter 2**.

4.2.3. Conditioned Pain Modulation (CPM) testing protocol

The cold pressor test and pressure pain threshold test were used to test the CPM effect as described in the literature[39,319]. The cold pressor test was used as the conditioning pain stimulus, which induces pain and triggers the CPM response followed by the PPT. The site of clinically determined (by palpation) maximal pain/tenderness in the mid-portion of the affected tendon in the tendinopathy group, and at the mid-portion of the tendon in controls was marked and used as the site for the pressure pain threshold testing as in previous studies[313,554]. Participants with tendinopathy were then asked to immerse the hand contralateral to the tendinopathy in a container with an ice-water slurry (at 5°C)[555] up to their wrists with their fingers abducted. The controls were asked to immerse their dominant hands and those with bilateral tendinopathy immersed the hand contralateral to the worse tendon. The participants were requested to inform the researcher when the cold induced pain of at

least 4 on the numerical pain rating scale after which the second pain stimulus (pressure pain) was applied on the tendon.

Pressure pain was applied using a digital pressure algometer (Type II, Somedic AB, Stockholm, Sweden) as validated in previous studies[39,319]. This was performed with the participants lying prone with the foot hanging at the edge of the plinth. The pressure from the 1cm^2 probe was applied perpendicularly on the skin at a constant rate of 40kPas^{-1}. The participants pushed a button to stop as soon as the stimulus changed from pressure to pain. Three consecutive readings were taken per participant (with one minute pauses in between) and were taken for analysis if the differences between them were below 100kPa, otherwise the process was repeated until the differences were <100kPa for a maximum of five times[39]. Prior to the actual test, to familiarise the participants with the PPT measurements, PPTs were performed over the left infraspinatus muscle before the actual experiment started (values not recorded).

4.2.4. Statistical analyses

The statistical analyses were conducted using the same statistical approach and tests as described in *Section 3.2.4* of **Chapter 3** for complementary types of data in this chapter.

4.3. RESULTS

Two-hundred and thirty participants were recruited for this study, of which seven (3 CON and 4 TEN) were excluded from the CPM analysis as they did not complete the cold pressor test. One-hundred CON and 123 TEN participants were therefore included in the analysis. The median duration of symptoms in the TEN group was 12 (IQR 6.0 - 36) months. There were no significant differences in sex, height, weight, dominant limb, years of participation in sport and hours of training per week during the previous two years between CON and TEN participants (Table 4.1). The TEN group was however significantly older, had a higher BMI and were mainly runners when compared to the controls (Table 4.1).

Participants with tendinopathy had a lower PPT (kPa) before [median (IQR)] [TEN: 417 (364 - 516) vs CON 601 (459 - 724), $p<0.001$] and during [TEN: 458 (358 - 550) vs CON 633 (506 - 753), $p<0.001$] the cold pressor test (Figures 4.1A and 4.1B). The mean pressure pain thresholds were higher during the cold pressor test in both groups ($p<0.001$). However, there was no difference in the CPM effect between the two groups; [median (IQR)] [TEN: 34.0 (-2 - 79) vs CON: 44.5 (3.5 - 94)] ($p=0.490$) (Figure 4.1C). Observed differences in CPM effect remained statistically insignificant after controlling for age, BMI, and main sport ($p=0.831$).

Table 4.1: Demographic characteristics of healthy controls without a history of Achilles tendinopathy (CON) and participants with a present history of chronic Achilles tendinopathy (TEN).

	CON (n=100)	TEN (n=123)	p-value
Age (years)	37 (31 - 50)	46 (36 - 52)	0.008
Sex (% male)	64 (64)	61 (75)	0.678
Height (cm)	174 ± 9	173 ± 9	0.419
Mass (kg)	71 (60 - 80)	75 (65 - 85)	0.073
BMI	23 (21 - 26)	25 (23 - 28)	0.001
Dominant leg (% right) †	88 (87)	77 (89)	0.104
Main sport (% running) ‡	63 (61)	82 (93)	0.003
Duration of participation in main sport (years)	10 (5.0 - 19)	9.0 (5.0 - 20)	0.674
Training (hours/week)			
Past 3 months	5.0 (3.0 - 7.3)	5.0 (3.0 - 7.0)	0.884
Past 12 months	5.0 (3.0 - 7.0)	6.0 (4.0 - 7.5)	0.278
Past 24 months	5.0 (3.0 - 6.0)	6.0 (3.0 - 8.0)	0.270

Height is expressed as average ± standard deviation, while sex, dominant limb and main sport are represented as a percentage with n in parentheses. The remaining variables are expressed as median (IQR).
† Ambidextrous participants: 4.0% (n=4) were CON and 8.6% (n=10) were TEN
‡ Other sports that the participants took part in: CON: 3.1% (n=3) cricket/golf; 11.3% (n=11) cycling; 3.1% (n=3) gym; 5.2% (n=5) hiking; 13.4% (n=4) other and TEN: 3.5% (n=4) cricket/golf; 0.9% (n=1) cycling; 4.4% (n=5) gym; 2.6% (n=3) triathlon; 7.0% (n=8) other

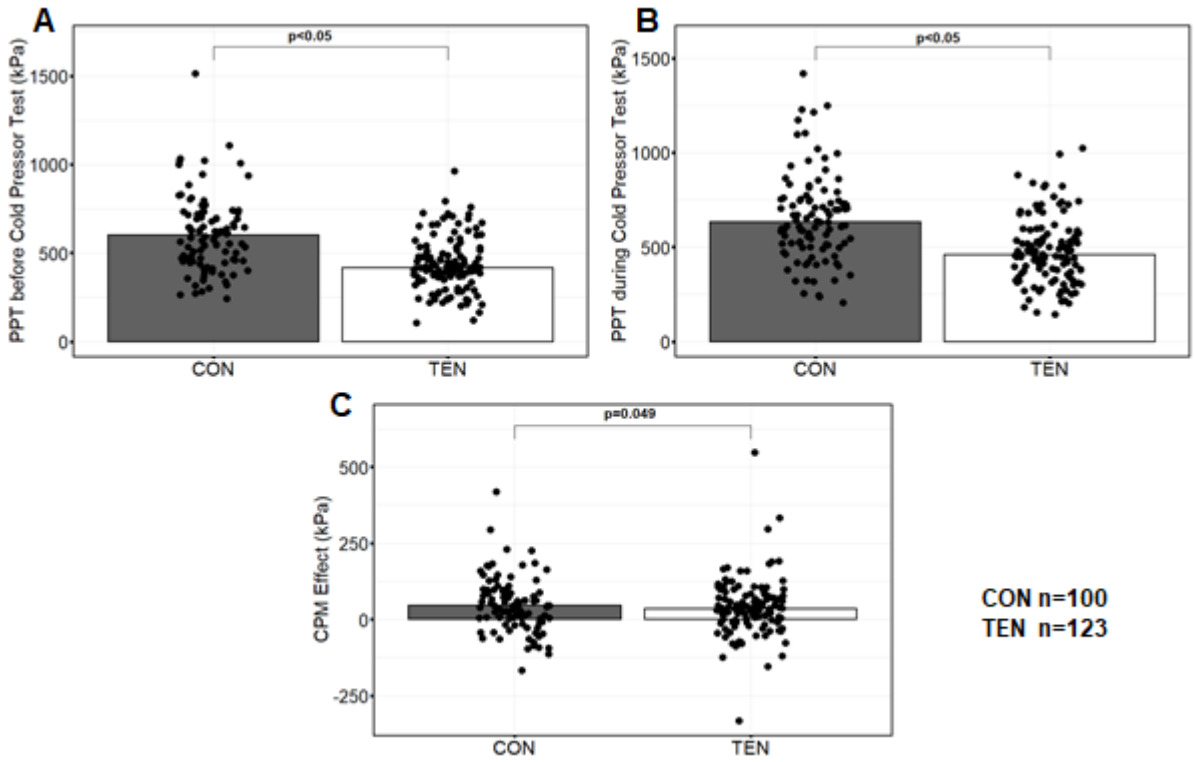

Figure 4.1: Pressure pain thresholds (PPT) **(A)** before and **(B)** during the cold pressor test and the **(C)** conditioned pain modulation (CPM) effect measured in kPa in individuals with chronic Achilles tendinopathy (TEN, n=123) and those without a previous history of Achilles tendinopathy (CON, n=100). Data are presented as medians and IQR with the values for individual participants indicated.

No significant independent associations were observed between sex (p=0.720), age (p= 0.209), BMI (p=0.058), duration of symptoms (p=0.558), self-reported pain scores and the CPM effect in the participants with tendinopathy (Table 4.2).

Table 4.2: Correlations between the conditioned pain modulation effect and pain scales indices from the Victorian Institute of Sports Assessment – Achilles questionnaire (VISA-A), short form of the McGill pain questionnaire (sf-MPQ) and short form of the Brief Pain Inventory (sf-BPI) in participants with chronic Achilles tendinopathy (TEN).

	Spearman's r (n=121)	95 % CI	p-value (adjusted p-value)†
VISA-A			
Total score	-0.02	-0.22 to 0.18	0.852 (0.425)
Sensory index	-0.03	-0.23 to 0.17	0.741 (0.312)
Interference index	0.06	-0.13 to 0.26	0.487 (0.920)
sf-MPQ			
Total score	-0.11	-0.29 to 0.07	0.218 (0.973)
Sensory index	-0.04	-0.22 to 0.14	0.656 (0.914)
Affective index	-0.08	-0.26 to 0.10	0.384 (0.414)
sf-BPI			
Total score	-0.15	-0.33 to 0.02	0.095 (0.599)
Intensity index	-0.11	-0.29 to 0.06	0.220 (0.563)
Interference index	-0.16	-0.33 to 0.01	0.077 (0.412)

† Adjusted for BMI

When considering the intensity of the seven most commonly chosen sf-MPQ word descriptors **(Chapter 2)** and the PPT before the cold pressor test, participants who described their pain as severe throbbing had lower PPT before the cold pressor test when compared to those whose pain was mild or moderate throbbing or who used the other six descriptors (p=0.016; Table 4.3). When considering the common pain descriptors reported by the participants on the PPI scale of the sf-MPQ, there were no differences between the pressure pain threshold [median (IQR)] before the cold

pressor test in participants who reported no pain [462 (381-593), n=41] compared to those with mild pain [416 (351-441), n=28, p=0.522] and between those who reported no pain [462 (381-593), n=41] compared to those with discomforting pain [409 (300-463), n=42, p=0,057]. Similarly, no significant correlations were observed between the pressure pain threshold before the cold pressor test [median (IQR)] and the VAS score of the sf-MPQ (Spearman's r = 0.173, p=0.059, n=120).

From the sf-BPI, PPT before the cold pressor test were significantly lower in individuals who reported mild to severe interferences in mood (p=0.023), general activity (p=0.038) and walking ability (p=0.004, Table 4.4) when compared to those who reported no interferences. Pressure pain thresholds before the cold pressor test were also significantly lower in those participants who reported mild to severe pain at the time of testing (p=0.024) or reported moderate to severe pain on average (p=0.014, Table 4.4).

Additionally, there were no associations between the intensity of the seven most commonly chosen sf-MPQ word descriptors and the CPM effect (Table 4.5). There were also no differences between the CPM effect [median (IQR)] in participants who reported no pain [34kPa (3-73), n=41] compared to those with mild pain [28kPa (-18-72), n=29, p=0.472] and between those who reported no pain [34kPa (3-73), n=41] compared to those with discomforting pain [38 (-9-98), n=42, p=0.897].

Table 4.3: The relationship between the median (IQR) pressure pain threshold (PPT) before the cold pressor test (kPa) and self-reported pain severity (none, mild, moderate and severe) for the seven most commonly used pain descriptors within the sensory index of the short form McGill Pain Questionnaire (sf-MPQ).

	None	Mild	Moderate	Severe	p- value
Aching	400 (351-491), 47	417 (341-549), 41	442 (388-618), 27	449 (238-506), 5	0.285
Tender	415 (359-534), 57	389 (337-464), 27	453 (395-604), 24	367 (251-470), 12	0.646
Throbbing	428 (378-579), 77	393 (274-464), 25	424 (350-471), 8	369 (240-494), 10	**0.016**
Sharp	420 (364-535), 78	430 (273-524), 17	400 (289-524), 17	429 (375-473), 8	0.478
Stabbing	424 (371-524), 85	393 (353-472), 13	477 (304-623), 13	295 (261-464), 9	0.246
Shooting	423 (370-515), 90	371 (259-424), 11	420 (230-665), 13	410 (331-497), 6	0.373
Hot burning	419 (369-519), 90	393 (318-543), 15	415 (183-494), 9	431 (333-520), 6	0.303
Continuous [a]	457 371-509), 16	398 (336-482), 46	422 (385-603), 41	388 (263-478), 17	0.624
Intermittent [b]	436 366-548), 58	405 (360-470), 22	420 244-618), 23	388 (388-451), 17	0.234
All [c]	463 412-508), 8	398 (335-486),40	443(378-586), 46	393 (294-458), 26	0.285

The number of participants (n) in each group is indicated after the median (IQR) values; p-values (none vs mild, moderate plus severe).
[a] Aching, Tender and Throbbing; [b] Sharp, Stabbing and Shooting; [c] all seven descriptors
Participants selected none for all of the individual descriptors to be included in the none group of the continuous, intermittent or all groups. For the mild, moderate and severe groups, at least one of the descriptors was described with a maximum pain of mild, moderate or severe, respectively.

Table 4.4: The relationship between the median (IQR) pressure pain threshold (PPT) before the cold pressor test (kPa) and the level of self-reported interference severity (none, mild, moderate and severe) of tendon pain for the domains of the short form Brief Pain Inventory (sf-BPI).

	None	Mild	Moderate & Severe	p-value
Pain interference domain				
Relationships	415 (370-532), 90	423 (230-525), 22	424 (239-480),7	0.542
Sleep	425 (375-537), 78	416 (252-479), 33	384 (294-439), 8	0.105
Mood	445 (386-541),56	404 (284-487), 44	398 (248-476), 20	**0.023**
General activity	436 (387-563), 54	408 (334-476), 51	364 (271-452), 14	**0.038**
Enjoyment of life	429 (364-548), 54	409 (350-488), 48	424 (319-472), 17	0.347
Normal work	442 (370-566), 53	412 (362-476), 54	400 (274-473), 12	0.162
Walking ability	490 (393-608), 32	409 (362-479), 66	388 (277-468), 21	**0.004**
Pain intensity domain				
At present	462 (387-605), 39	413 (322-510), 68	380 (253-445), 12	**0.024**
On average	527 (413-635), 4	424 (378-536), 85	383 (249-455), 30	**0.014** [a]
At its least	428 (384-577), 42	415 (294-519), 66	388 (266-452), 11	0.190
At its worst	421 (372-528), 64		415 (282-515), 55	0.517 [a]

The number of participants (n) in each group is indicated after the median (IQR) values; p-values (none vs mild, moderate plus severe).
[a] None plus mild vs moderate plus severe

Table 4.5: The relationship between the median (IQR) conditioned pain modulation (CPM) effect (kPa) and self-reported pain severity (none, mild, moderate and severe) for the seven most commonly used pain descriptors within the sensory index of the short form McGill Pain Questionnaire (sf-MPQ).

	None	Mild	Moderate	Severe	p- value
Aching	18 (-11 - 67), 47	52 (12 - 93), 42	39 (-10 - 57), 27	36 (1 - 42), 5	0.091
Tender	32 (-11 - 82), 58	43 (18 - 79), 27	37 (-10 - 91), 24	29 (-27 - 45), 12	0.646
Throbbing	34 (-11 - 83), 78	43 (20 - 100), 25	-1 (-73 - 36), 8	32 (-22 - 54), 10	0.968
Sharp	37 (-2 - 85), 79	36 (2 - 75), 17	8 (-59 - 83), 17	40 (31 - 132), 8	0.529
Stabbing	34 (-11 - 80), 86	72 (30 - 11), 13	17 (-14 - 57), 13	34 (28 - 78), 9	0.254
Shooting	41 (-2 - 90), 91	27 (16 - 45), 11	17 (-77 - 107), 13	-6 (-35 - 36), 6	0.077 0.060 [d]
Hot burning	40 (-2 - 90), 91	29 (-9 - 57), 15	33 (-9 - 100), 9	20 (-13 - 40), 6	0.407
Continuous [a]	3 (-32 - 64), 16	43 (15 - 81), 47	34 (-13 - 91), 41	34 (-25 - 74), 17	0.171
Intermittent [b]	37 (-9 - 85), 59	38 (11 - 72), 22	30 (-72 - 107), 23	34 (13 - 78), 17	0.904
All [c]	11 (-18 - 49), 8	41 (9 - 77),41	37 (-30 - 99), 46	34 (-13 - 67), 26	0.322

The number of participants (n) in each group is indicated after the median (IQR) values; p-values (none vs mild, moderate plus severe).
[a] Aching, Tender and Throbbing; [b] Sharp, Stabbing and Shooting; [c] all seven descriptors
Participants selected none for all of the individual descriptors to be included in the none group of the continuous, intermittent or all groups. For the mild, moderate and severe groups, at least one of the descriptors was described with a maximum pain of mild, moderate or severe, respectively.
[d] none plus mild vs moderate plus severe.

Similarly, no significant correlations were observed between the CPM effect and the VAS score of the sf-MPQ (Spearman's r = -0.032, p=0.731, n=121). However, from the sf-BPI, a low CPM effect was significantly associated with mild or severe interference with sleep (p=0.043) (Table 4.6). There was no other significant difference between the CPM effect among the severity groups of the activities reported on the sf-BPI pain interference domain or any of the four pain intensity domain descriptors (Table 4.6).

Table 4.6: The relationship between the median (IQR) conditioned pain modulation (CPM) effect (kPa) and the level of self-reported interference severity (none, mild, moderate and severe) of tendon pain for the domains of the short form Brief Pain Inventory (sf-BPI).

	None	Mild	Moderate & Severe	p-value
Pain interference domain				
Relationships	37 (-2-81), 91	31 (-19-89), 22	30 (-20-34), 7	0.412
Sleep	40 (3-91), 79	28 (-63-58), 33	25 (-6-43), 8	**0.043**
Mood	37 (-1-78),57	42 (6-106), 44	29 (-38-41), 19	0.936
General activity	42 (-9-91), 55	34 (3-90), 51	29 (-22-37), 14	0.497
Enjoyment of life	42 (4-91), 54	35 (-10-97), 48	29 (-18-41), 17	0.313
Normal work	50 (3-98), 54	32 (-2-86), 54	29 (-37-38), 12	0.119
Walking ability	37 (-6-86), 33	40 (-2-98), 66	30 (-13-42), 21	0.952
Pain intensity domain				
At present	37 (-2-81), 39	39 (-2-88), 69	29 (-27-63), 12	0.810
On average	48 (-17-69), 4	37 (-9-90), 86	32 (8-58), 30	0.944 [a]
At its least	56 (2-101), 42	30 (-2-67), 67	30 (-29-72), 11	0.159
At its worst	40 (-2-92), 65		30 (-9-68), 55	0.358 [a]

The number of participants (n) in each group is indicated after the median (IQR) values; p-values (none vs mild, moderate plus severe).
[a] None plus mild vs moderate plus severe

4.4. DISCUSSION

The main findings of this study were that (i) recreational athletes with AT had lower PPT when compared to pain free controls, (ii) there was no difference in the CPM effect between athletes with AT compared to healthy controls, (iii) participants who reported severe throbbing pain, (iv) mild to severe interferences in mood, walking ability and general activity and (v) mild to severe pain at the time of testing or moderate to severe pain on average had lower PPT before the cold pressor test. Additionally, in this study low CPM effect was associated with moderate to severe interference with sleep.

The results of this study are contrary to those of Tompra et al.[39] and Rathleff et al[553] who observed a lower CPM effect in individuals with chronic AT and patellofemoral joint pain, respectively. However, the results of the present study are consistent with data obtained by Plinsinga and colleagues[132] and Rathleff et al[556] who noted that AT and PT and patellofemoral join pain, respectively, appear to not have features of central sensitisation. Central sensitisation has however been observed consistently in individuals with LET[133] and in other cohorts of patellofemoral joint pain[557]. It has also been observed in individuals with various other conditions such as chronic pelvic pain, headaches, and IBS[311,318]. Although it is tempting to draw direct parallels with these other conditions, given the different types of tissue and loads that these structures experience and the differences in pathologies (neuropathic, musculoskeletal, visceral) of the conditions, tendon pain literature suggests that such comparisons would be tenuous at best[347].

However, pain disorders are often comorbid and share similar biopsychosocial risk profiles for pain and activity impairment[44]. Moreover, research in other medical specialties (dentistry, rheumatology, gastroenterology, nursing, gynaecology, and neurology) has also shown that the pathways involved in central sensitisation and pain amplification are similar across pain syndromes[328,558]. Fibromyalgia[559], OA[560], whiplash, LBP[320,561], chronic pancreatitis[329,562] and distal oesophageal pain[563] show evidence of central sensitisation and impairments in inhibitory pain modulation[564]. Similar to tendinopathy, there is also poor correlation between peripheral tissue abnormalities and self-reported pain in these conditions. This suggests that altered central pain processing and abnormal endogenous pain modulation is a shared characteristic of many chronic pain conditions[316]. Hence, treatment paradigms would need to be broadened from the historical tendon pathology focus and to consider chronic tendon pain as a condition of the nervous system as well as that of the tendon. However, translation of these findings to practice is difficult as CNS involvement in chronic musculoskeletal pain is not a constant finding.

Studies on CPM in chronic LBP have produced mixed results where the reduced CPM effect is not a constant finding in different cohorts of cases and controls[319,321,323]. As with LBP, AT seems to be a very heterogeneous condition, which may explain the variability in literature with regards to CPM. It is possible that a subset of individuals with Achilles tendinopathy display such altered central pain processing, but in the absence of normative data it is hard to identify them more specifically and thus individualise their therapy to include desensitisation treatments. Unlike LBP and LET, however, there is a lack of studies on CPM in AT with which to compare. This is further complicated by variations in aspects of CPM methodology and paradigms across the

literature as seen in the present compared with Tompra et al.[39] This limits direct comparisons and could explain the contrasting results.

There are a number of differences in methodology across studies on CPM. Different inclusion criteria, temperatures, testers, paradigms, type of and location of stimuli and type of participants[554,555,565,566] have been employed in literature and these can be potential confounders to the results obtained with CPM experiments[347]. Experts are confident that CPM methodology is an invaluable tool for assessing central pain mechanisms in various diseases[566], however, they also acknowledge that currently no CPM methodology is the gold standard[567]. With that, the need to optimise and standardise the procedure is imperative in order to allow direct comparisons across cohorts and better understand the role of central pain mechanisms in disease states[568].

Another novel finding of this study was that lower PPT scores were observed in AT especially in those who had mild to severe pain at the time of testing, described their pain as severe throbbing or had mild to severe interferences with general activity and walking ability. This may suggest a peripheral mechanical hyperalgesia as a characteristic of chronic tendinopathies such as AT[569,570], LET[571], adductor longus[572], PT[132] as well as other lower limb maladies such as chronic patellofemoral pain[557,573]. This could be facilitated by locally produced algogenic substances such as SP from tenocytes[574]. This assertion concurs with results from a previous Achilles tendinopathy cohort[132] but not from others[39] and may support a role of the peripheral nociceptive input as an important factor driving the development of spreading sensitisation. While hyperalgesia is considered as one of the characteristics of central sensitisation[575], the

present results seem to suggest a more peripheral source of pain. This is at odds with clinical practice where peripheral, tissue-based treatments are not very effective[87]. The most efficacious treatment for tendinopathy to date is exercise[550,551,576], whose long-term analgesic effects are believed to involve activation of the descending inhibitory pain pathway(s)[568]. Exercise therapy has been proposed as a 'desensitising' treatment for conditions whose pain is suspected to be of a central nature. Its purported mechanism of action is by altering pain memories in patients with chronic musculoskeletal pain[577,578], which may explain the association with mood in this cohort. Therefore, though currently elusive, it is possible that there is a CNS driver for tendon pain as demonstrated in numerous other pain conditions[579].

A novel finding of this study was that a lower CPM effect was associated with interferences in sleep among athletes with tendinopathy. This phenomenon has been observed in other pain conditions[580–582] but to the author's knowledge, this is its first documentation in chronic AT. Chronic pain patients, tendinopathy included, suffer from sleep disturbances **(Chapter 2),** which have been shown to lead to impairments in CPM, facilitation of temporal summation and increased pain sensitivity[581,583,584]. Therefore, assessing sleep as well as providing sleep therapy as part of rehabilitation of tendinopathy may be important to alleviate some of the effects of chronic tendon pain. This is an assertion supported by results that show that good quality sleep is associated with greater pain modulation[585]. However, a major limitation of these studies and the results thereof is that they are cross sectional in nature, which makes it difficult to determine causation; pain disrupts sleep and impaired sleep disrupts pain modulation[580]. It may still be worthwhile, however, to break the cycle by targeting either one to improve the other.

The present results also did not show a relationship between pain intensity scores and the CPM effect unlike in studies of other chronic pain conditions[330]. From the present cohort, despite the median duration of symptoms being rather long, the VISA-A scores were high and the participants in this cohort continued to train even though they had tendon pain and learnt to manage it; 'it will not kill me', 'it's more of an annoyance', 'I put it at the back of my mind' (personal communication, 2019). This finding is not uncommon in athletes as they have been shown to have lower pain intensity ratings and higher pain tolerance when compared to non-athletes[508,586,587]. This is likely a result of physiological and psychological coping mechanisms developed in training[509].

Physiological adaptations such as the cross-stressor hypothesis and the changes to the catecholaminergic and HPA as a result of repeated exercise training may explain why athletes seem to manage pain such Achilles tendon pain differently from other chronic pain states. According to this hypothesis, the repeated physiological insult of regular exercise results in adaptations, which lead to reduced sensitivity to homotypic (more exercise) and heterotypic stressors (other stressors) such as pain in this instance[588] as demonstrated by reduced catecholaminergic and HPA responses to stressors[589]. Therefore, it is reasonable to assume that as AT is often a result of regular participation in exercise endeavours, these participants' continued training has led to altered adrenal and HPA function.

This study is not without limitations though. While the present cohort was larger than previous ones, it was a small percentage of the potential population, therefore these results should be generalised to recreational athletes with caution. Additionally, this

cohort is made up of runners who were training for a particular race and had not stopped their training on account of the pain. Therefore, the responders to the research call could represent a particular personality type and with the link between pain and personality well established[519–521,590,591], perhaps studying the extremes of the phenotype may provide more data on CPM in tendinopathy. Thirdly, although CPM is generally used as a proxy to assess central pain processing, using functional MRI may have yielded more accurate results in showing differences in central pain processing[592]. Additionally, the assessor was not blinded, this may have introduced bias into the testing procedures.

In conclusion, there was no difference in the CPM effect between athletes with tendinopathy and controls in this study, which implies that there are no alterations in descending pain modulation with Achilles tendinopathy. However, perhaps this result can be explained by the altered pain perception that may result from changes in the functions of the HPA as a consequence of repeated exercise training. Additionally, there is a need for more studies on CPM in AT in order to improve comparisons across the studies and allow a better understanding of the relationship between CPM and AT.

CHAPTER FIVE

GENETIC VARIATION AND SUSCEPTIBLITY TO PAIN

A condensed version of this chapter has been submitted for peer review as:

Mkumbuzi N.S., Dlamini S.B., Mafu T.S., September A.V., Posthumus M. & Collins M. A novel evaluation of *COMT, SCN9A, TAC1* and *TACR-1* polymorphisms in a South African cohort of recreational athletes with chronic Achilles tendinopathy highlights an association between *COMT*, pain sensitivity and self-reported tendon pain. **Under review**

5.1. INTRODUCTION

As discussed in previous chapters, the cardinal symptom of chronic AT is pain, which has an insidious onset and is associated with mechanical loading but improves with continued movement[106]. Although the aetiology of chronic AT is poorly understood, it is well established that common DNA sequence variants modulate risk of injury[593]. Previous research has focused on variants within genes that encode the structural components of the ECM or regulate biological processes within tendon and ligament[356,380,594].

Genetic variants, which have previously been associated with pain perception, modulation, transduction, transmission and conduction[47], could modulate the inter-individual variability in the pain experiences during chronic AT. These associations may, in part, contribute to the current poor association between tendon pathology, pain and disability[13] **(Chapter 2).** To the author's knowledge, the association of genetic variants with pain has not been previously investigated in chronic painful AT. However, this has been extensively investigated in other conditions, which has contributed to elucidating the mechanisms of pain, informing treatment options[46,47,595].

At a biochemical level, neuropeptides such as SP have been hypothesised to stimulate peripheral nociceptors and eventually lead to pain[596]. As discussed in *Section 1.5.1.2* of **Chapter 1**, SP has a well-known nociceptive role in the central nervous system[220] and, together with its receptor, has been observed in symptomatic tendinopathy[220,243,597]. As the expression of NK-1R in pathological tendons can be considered to mirror that of SP, the latter's importance in tendinopathy can also be inferred. Although variants in genes encoding SP (*Tac1*) and NK-1R (*TACR1*) have not been widely associated with human musculoskeletal pain phenotypes, they have been associated with mood and affective disorders[598]. Because of the proposed importance of SP and its receptor in tendinopathy[599] and the effect of tendon pain on affect[99], they were included in this study.

As discussed in more detail in *Section 1.5.4* of **Chapter 1**, *COMT* polymorphisms are associated with a number of pain syndromes such as Parkinson's disease (PD) related pain[440], linked to the development of chronic postoperative pain[600], variability in OA pain[444] and lower pain related disability in LBP[457]. Additionally, as reviewed in *Section 1.5.4* of **Chapter 1**, polymorphisms in ion channel genes such as *SCN9A* have been associated with altered resting membrane potential in DRG neurons. In particular, the rs6746030 polymorphism also doubled the firing frequency and thus affected susceptibility to pain in a mixed cohort of participants with sciatica, OA and pancreatitis among others[397,398]. Furthermore, the rs6746030 polymorphism was also shown to contribute to the risk or severity of post-operative pain[601], bladder pain[602] and basal pain (pressure and heat pain)[603]; the minor A allele was associated with increased pain scores[399].

Therefore, the aim of this chapter was to determine the association between *COMT* rs4818 (C/G), *COMT* rs4633 (C/T), *TAC1* rs2072100 (C/T), *TACR1* rs3771829 (C/G) *and SCN9A* rs6746030 (G/A) polymorphisms with pain sensitivity as well as self-reported pain in a cohort of recreational South African runners with chronic painful Achilles tendinopathy. Based on previous findings on pain, it was hypothesised that *COMT* rs4818 CC[442], the rs4633 TT[441] and the *SCN9A* rs6746030 GG[399] genotypes would be associated with higher pain sensitivity and higher pain scores.

5.2. MATERIALS AND METHODS

5.2.1. Participants and pain self-report

The 129 recreational athletes with chronic AT (TEN) included in this chapter of the thesis were recruited as described in *Section 2.2.1* of **Chapter 2.**

The participants completed the self-administered eight question VISA-A (Appendix F), the sf-MPQ (Appendix G) and the sf-BPI (Appendix H), as previously described in *Section 2.2.2* of **Chapter 2.**

5.2.2. Conditioned pain modulation (CPM) protocol

The cold pressor test and pressure pain threshold test were used to test the CPM effect as previously described in *Section 4.2.3* of **Chapter 4**.

5.2.3. DNA extraction and genotyping

Approximately 5ml of venous blood was collected by a trained phlebotomist (Nonhlanhla Mkumbuzi) from the antecubital vein of the non-dominant forearm of each participant and stored in an EDTA vacutainer tube at -20°C until total genomic DNA was extracted as previously described[604].

Standard TaqMan™ Genotyping Assays (Applied Biosystems, Foster City, CA, USA) were used to genotype the DNA samples for *COMT* rs4818 (C/G), *COMT* rs4633

(C/T), *Tac 1* rs2072100 (C/T), *TACR1* rs3771829 (C/G) and *SCN9A* rs6746030 (G/A). The PCR reactions were conducted using the Applied Biosystems QuantStudio™ Real-Time PCR system (Applied Biosystems) following the manufacturer's recommendations. Negative controls (no DNA) and five repeat samples (known genotypes) were included on every PCR plate as a quality control measure for reliable genotyping and detection of any possible contamination. Genotypes were called automatically using the Thermo Fisher Cloud suite version 3.3.0-SR2-build 21 (Thermo Fisher Scientific, Foster City, CA, USA) with an average 98.0% call rate. All laboratory work was conducted at the Division of Exercise Science and Sports Medicine laboratories at the University of Cape Town.

5.2.4. Statistical analyses

In general, statistical tests and level of significance were as stated previously in *Section 3.2.4* of **Chapter 3**. In addition, differences in genotype groups were analysed using Statistica® v13.2 (Tulsa, OK, USA) and R studio version 1.2.1335, running R statistical software version 3.6.1 (Foundation for Statistical Computing, Vienna, Austria). Hardy–Weinberg equilibrium (HWE) was calculated using 'HardyWeinberg' version 1.6.3. package. For post-hoc analyses, the χ^2 test was used for categorical data and the Kruskal- Wallis associated multiple comparisons test with Bonferroni adjustment was used for quantitative data.

5.3. RESULTS

One hundred and twenty-nine (129) participants with chronic AT were included in this study: 61.2% (n=79) were male and 61.2% (n=79) described themselves as Caucasian and 38.8% (n=50) where of various ethnicities. At least one SNP was successfully genotyped for each included participant. The genotype distributions of all the SNPs within all participants and the Caucasian sub-group were in HWE (Table 5.1). Since similar genotype and allele distributions were observed among the population sub-groups (Table 5.1) and population stratification is not a consideration for the study design, all participants were combined for further analysis.

With two exceptions, there were no significant differences in the demographic and injury characteristics as well as sporting history and training duration during the last 24 months for the different genotype groups of the five investigated SNPs (p>0.050) (Tables 5.2-5.6). There was a significant difference in the number of runners among the *COMT rs4633* genotype groups (CC: 88.5%, n=23; CT: 84.9%, n=45 and TT: 72.7%, n=24; p=0.035; Table 5.3) and the duration of chronic AT symptoms in the *TACR1 rs3771829* genotype groups [median (IQR)-CC:12.0 (6.0 – 39.0) n=97; CG: 10.0 (3.0 – 19.0) n=26; p=0.019; Table 5.5].

Table 5.1: Genotype and minor allele frequencies (MAF) for all participants (n=129) with chronic Achilles tendinopathy, the self-reported Caucasian sub-group (n=79) and the self-reported non-Caucasian sub-group (n=50).

SNP and Groups	n	Genotype (%)			p-value	HWE	MAF (%)
***COMT* rs4818**		**CC**	**CG**	**GG**			**G**
All participants	123	52.0	40.7	7.3		0.857	27.6
Caucasian Sub-Group	76	48.7	42.1	9.2	0.459 [a]	0.983	30.3
Non-Caucasian Sub-Group	47	57.4	38.3	4.3		0.257	23.4
***COMT* rs4633**		**CC**	**CT**	**TT**			**T**
All participants	124	22.6	50.0	27.4		0.979	53.1
Caucasian Sub-Group	74	17.6	50.0	32.4	0.155	0.846	57.4
Non-Caucasian Sub-Group	50	30.0	50.0	20.0		0.935	45.0
***TAC1* rs2072100**		**CC**	**CT**	**TT**			**C**
All participants	129	26.4	50.4	23.2		0.920	51.6
Caucasian Sub-Group	75	25.3	54.7	20.0	0.453	0.404	52.7
Non-Caucasian Sub-Group	50	28.0	44.0	28.0		0.140	50.0
***TACR1* rs3771829**		**CC**	**CG**	**GG**			**G**
All participants	125	78.4	21.6	0.0		0.176	10.8
Caucasian Sub-Group	75	77.3	22.7	0.0	0.093 [a]	0.268	11.3
Non-Caucasian Sub-Group	50	90.0	10.0	0.0		0.462	10.0
***SCN9A* rs6746030**		**GG**	**GA**	**AA**			**A**
All participants	121	75.2	23.9	0.9		0.707	10.7
Caucasian Sub-Group	72	76.4	23.6	0.0	0.369 [b]	0.256	11.8
Non-Caucasian Sub-Group	49	83.7	14.3	2.0		0.130	9.2

MAF is defined at the reported allele within the European population within the ENSEMBL genome database.
p-values were determined by comparing the genotype distributions of the Caucasian and non-Caucasian population groups.
[a] CC vs CG + GG, [b] GG vs GA + AA
Missing genotype data for all participants: 5 (3.9%) *COMT* rs4633 (C/T), 6 (4.7%) *COMT* rs4818 (G/C), 4 (3.1%) *TACR1* rs3771829 and 8 (6.2%) *SCN9A* rs6746030 (A/G).

Table 5.2: *COMT* rs4818 (G/C) genotype distributions for all the participants with chronic Achilles tendinopathy (AT) (i) demographic and injury characteristics, (ii) sporting history and training duration during the preceding 24 months, (iii) self-reported tendon pain from the short form McGill Pain Questionnaire (sf-MPQ), short form Brief Pain Inventory (sf-BPI) and the Victorian Institute Sports Assessment – Achilles (VISA-A), (iv) pressure pain thresholds (PPT) before (A) and during (B) ice immersion, and (v) conditioned pain modulation (CPM).

	CC (n=61)	**CG** (n=50)	**GG** (n=7)	**p-value**
Age at recruitment (years)	47.9 ± 10.3	44.3 ± 11.6	44.0 ± 10.2	0.674
Sex (% male)	37.5	64.0	61.9	0.366
Height (cm)	170.4 ± 10.6	172.9 ± 8.6	173.2 ± 9.4	0.750
Weight (kg)	67.4 ± 11.3	75.2 ± 14.2	76.3 ± 13.9	0.282
BMI ($kg.m^{-2}$)	21.5 (20.4 – 27.2)	24.0 (23.0 – 27.4)	24.9 (22.7 – 28.0)	0.355
Dominant limb (% right)†	71.4	76.6	76.3	0.910
Bilateral AT (%)	14.3	34.0	33.3	0.586
Unilateral AT (% right)	28.6	40.0	38.3	0.586
Duration of symptoms (months)	12.0 (4.0 – 18.0)	12.0 (6.0 – 36.0)	12.0 (94.0 – 30.0)	0.337
Main Sport (% running)‡	100	84.4	76.3	0.499
Participation in main sport (yrs)	20.0 (8.0 – 24.0)	11.0 (5.0 – 20.0)	9.0 (4.0 – 20.0)	0.455
Training 1 to 3 months (hrs/wk)	5.0 (1.0 – 6.0)	6.0 (2.5 – 7.0)	5.0 (3.0 – 8.0)	0.609
Training 4 to 12 months (hrs/wk)	6.0 (4.0 – 6.0)	6.0 (4.0 – 7.0)	6.0 (4.0 – 8.0)	0.579
Training 13 to 24 months (hrs/wk)	5.0 (1.0 – 7.0)	6.0 (2.5 – 7.0)	6.0 (4.0 – 8.0)	0.457
sf-BPI Total	15.0 (9.0 – 25.0)	17.0 (10.0 – 29.0)	16.0 (6.0 – 32.0)	0.912
sf-BPI Intensity Subscale	10.0 (6.0 – 14.0)	10.0 (6.0 – 15.0)	10.0 (4.0 – 20.0)	0.972
sf-BPI Interference Subscale	6.0 (1.0 – 14.0)	6.0 (2.0 – 14.0)	6.0 (0.0 – 14.0)	0.854
VISA-A Total	68.6 ± 16.4	71.7 ± 15.9	70.4 ± 15.2	0.615
VISA-A Sensory Subscale	39.0 (32.0 – 45.0)	39.0 (33.0 – 47.0)	41.0 (35.0 – 46.0)	0.688
VISA-A Interference Subscale	33.0 (25.0 – 38.0)	34.0 (27.0 – 40.0)	36.0 (27.0 – 37.0)	0.558
PPT A	423 (295 – 543)	400 (371 – 462)	525 (398 – 595)	0.152
PPT B	431 (350 – 587)	455 (369 – 527)	554 (402 – 652)	0.394
CPM	28.0 (-2.0 – 77.0)	37.5 (1.0 – 90.0)	30.5 (-3.0 – 56.0)	0.493

Height is expressed as average ± standard deviation, while sex, dominant limb and main sport are represented as a percentage. The remaining variables are expressed as median (IQR).
† Ambidextrous participants: 6.8% (n=4), 10.6% (n=5), 14.3% (n=1) were *CC*, *CG* and *GG* genotypes, respectively.
‡ Other sports that the participants took part in: CC- 3.4% (n=2) cricket/golf; 1.7% (n=1) cycling; 8.5% (n=5) gym; 3.4% (n=2); 6.8% (n=4) other, CG – 2.2% (n=1) cricket/golf; 2.2% (n=1) cycling; 2.2% (n=1) triathlon; 8.9% (n=4) other.
CPM = PPT B minus PPT A

Table 5.3: *COMT* rs4633 (C/T) genotype distributions for all participants with chronic Achilles tendinopathy (AT) (i) demographic and injury characteristics, (ii) sporting history and training duration during the preceding 24 months, (iii) self-reported tendon pain from the short form McGill Pain Questionnaire (sf-MPQ), short form Brief Pain Inventory (sf-BPI) and the Victorian Institute Sports Assessment – Achilles (VISA-A), (iv) pressure pain thresholds (PPT) before (A) and during (B) ice immersion, and (v) conditioned pain modulation (CPM).

	CC (n=27)	**CT** (n=61)	**TT** (n=34)	**P-value**
Age at recruitment (years)	43.4 ±10.1	44.7 ± 11.0	45.5 ± 10.1	0.759
Sex (% male)	66.7	60.7	61.8	0.863
Height (cm)	173.5 ±10.8	172.8 ± 8.9	173.3 ± 8.3	0.937
Weight (kg)	77.3 ± 13.2	75.7 ± 14.3	73.6 ± 14.0	0.612
BMI ($kg.m^{-2}$)	25.6 (21.5 – 28.0)	24.6 (23.3 – 27.7)	24.0 (22.0 – 27.7)	0.749
Dominant limb (% right)†	76.9	80.4	75.0	0.268
Bilateral AT (%)	24.0	38.3	27.3	0.551
Unilateral AT (% right)	36.0	36.7	42.4	0.551
Duration of symptoms (months)	18.0 (5.0 – 36.0)	12.0 (6.0 – 36.0)	12.0 (4.0 – 36.0)	0.570
Main Sport (% running)‡	88.5	84.9	72.7	**0.035**
Participation in main sport (yrs)	10.5 (4.0 – 17.0)	11.0 (5.0 – 23.0)	8.0 (4.0 – 20.0)	0.679
Training 1 to 3 months (hrs/wk)	6.0 (3.0 – 7.0)	5.0 (3.0 – 7.5)	6.0 (3.5 – 7.5)	0.731
Training 4 to 12 months (hrs/wk)	6.0 (3.5 – 7.5)	6.0 (4.0 – 7.0)	6.0 (5.0 – 9.0)	0.438
Training 13 to 24 months (hrs/wk)	6.0 (2.5 – 8.5)	6.0 (3.8 – 7.5)	5.0 (3.0 – 8.0)	0.921
sf-MPQ Total	5.6 (3.3 – 10.5)	8.0 (4.0 – 12.0)	7.9 (4.0 – 13.0)	0.530
PPI	1.0 (0.0 – 1.0)	2.0 (0.0 – 2.0)	2.0 (0.0 – 2.0)	0.109
VAS	0.9 (0.0 – 2.3)	0.7 (0.0 – 3.5)	0.1 (0.0 – 3.5)	0.812
sf-MPQ Sensory Subscale	3.5 (2.0 – 6.0)	3.0 (2.0 – 6.0)	3.5 (2.0 – 7.0)	0.863
sf-MPQ Affective Subscale	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.0 (0.0 – 1.0)	0.346
sf-BPI Total	15.5 (9.0 – 29.0)	14.0 (9.0 – 27.0)	21.0 (11.0 – 31.0)	0.706
sf-BPI Intensity Subscale	9.5 (7.0 – 12.0)	9.5 (6.0 – 15.0)	11.0 (8.0 – 14.0)	0.780
sf-BPI Interference Subscale	7.0 (2.0 – 14.0)	4.5 (1.0 – 14.0)	10.0 (2.0 – 19.0)	0.761
VISA-A Total	72.4 ± 12.3	70.8 ± 16.4	66.1 ± 17.6	0.256
VISA-A Sensory Subscale	40.5 (36.0 – 46.0)	38.5 (31.0 – 47.0)	38.5 (33.0 – 45.0)	0.570
VISA-A Interference Subscale	34.5 (30.0 – 37.0)	34.0 (27.0 – 40.0)	29.0 (18.0 – 36.0)	0.118
PPT A	428 (318 – 535)	420 (374 – 513)	416 (283 – 464)	0.637
PPT B	456 (325 – 683)	464 (369 – 546)	430 (329 – 539)	0.681
CPM	43.0 (4.0 – 126)	30.0 (-16.0 – 72.0)	37.0 (6.0 – 79.0)	0.327

Height and total VISA-A score are expressed as average ± standard deviation, while sex, dominant limb and main sport are represented as a percentage. The remaining variables are expressed as median (IQR).
† Ambidextrous participants: 3.1% (n=1), 8.9% (n=5), 15.4% (n=4) were *TT*, *CT* and *CC* genotypes, respectively.
‡ Other sports that the participants took part in: *TT*- 6.1% (n=2) cricket/golf; 12.1% (n=4) gym; 6.1% (n=2) triathlon; 3.0% (n=1) other, *CT* – 3.8% (n=2) cycling; 1.9% (n=1) triathlon; 9.4% (n=5) other; *CC*- 3.9% (n=1); 3.9% (n=1) gym; 3.9% (n=1) other.
PPI – present pain intensity; VAS – visual analogue scale; CPM = PPT B minus PPT A

Table 5.4: *TAC1 rs2072100* (C/T) genotype distributions for all the participants with chronic Achilles tendinopathy (AT) (i) demographic and injury characteristics, (ii) sporting history and training duration during the preceding 24 months, (iii) self-reported tendon pain from the short form McGill Pain Questionnaire (sf-MPQ), short form Brief Pain Inventory (sf-BPI) and the Victorian Institute Sports Assessment – Achilles (VISA-A), (iv) pressure pain thresholds (PPT) before (A) and during (B) ice immersion, and (v) conditioned pain modulation (CPM).

	CC (n=33)	**CT** (n=57)	**TT** (n=29)	**P-value**
Age at recruitment (years)	45.0 ± 10.3	45.4 ± 10.7	41.3 ± 11.0	0.228
Sex (% male)	57.6	62.2	65.5	0.808
Height (cm)	171.6 ± 9.2	173.1 ± 8.5	174.8 ± 10.3	0.422
Weight (kg)	76.3 ± 14.8	76.4 ± 13.8	72.8 ± 12.8	0.502
BMI ($kg.m^{-2}$)	24.1 (21.9 -28.1)	25.5 (23.5 -27.8)	23.7 (22.7- 25.9)	0.336
Dominant limb (% right)†	78.1	77.2	73.1	0.967
Bilateral AT (%)	29.0	30.0	39.3	0.805
Unilateral AT (% right)	38.7	36.7	39.3	0.805
Duration of symptoms (months)	9.5 (4.0 – 36.0)	12.0 (6.0 – 36.0)	12.0 (6.0 – 38.0)	0.771
Main Sport (% running)‡	77.4	82.1	84.6	0.264
Participation in main sport (yrs)	8.0 (5.0 – 16.5)	12.0 (4.0 – 21.0)	14.0 (7.0 – 25.0)	0.221
Training 1 to 3 months (hrs/wk)	4.0 (2.0 – 6.0)	6.0 (4.0 – 8.0)	4.0 (2.0 – 7.0)	0.068
Training 4 to 12 months (hrs/wk)	5.0 (2.0 – 7.0)	6.0 (4.0 – 8.0)	6.0 (4.0 – 6.0)	0.241
Training 13 to 24 months (hrs/wk)	5.0 (2.0 – 7.0)	6.0 (3.0 – 8.0)	5.0 (3.0 – 7.0)	0.535
sf-MPQ Total	7.8 (4.0 – 13.2)	7.5 (4.0 – 11.0)	7.1 (3.0 – 12.0)	0.651
PPI	1.0 (0.0 – 2.0)	1.0 (0.0 – 2.0)	1.0 (0.0 – 2.0)	0.676
VAS	0.5 (0.0 – 3.5)	1.1 (0.0 – 3.3)	0.0 (0.0 – 3.1)	0.835
sf-MPQ Sensory Subscale	4.0 (2.0 – 8.0)	3.0 (2.0 – 6.0)	3.0 (1.5 – 6.0)	0.550
sf-MPQ Affective Subscale	0.0 (0.0 – 1.0)	0.0 (0.0 – 0.0)	0.0 (0.0 – 1.5)	0.271
sf-BPI Total	17.0 (7.0 – 32.0)	14.5 (10.0 – 25.0)	21.5 (8.5 – 30.5)	0.725
sf-BPI Intensity Subscale	8.0 (6.0 – 15.0)	10.0 (6.0 – 14.0)	11.5 (6.0 – 15.5)	0.688
sf-BPI Interference Subscale	7.0 (1.0 – 23.0)	4.0 (1.0 – 14.0)	7.5 (2.0 – 15.5)	0.446
VISA-A Total	71.5 ± 16.4	67.5 ± 16.0	73.5 ± 15.1	0.218
VISA-A Sensory Subscale	40.0 (35.0 -48.0)	38.0 (31.0 – 45.0)	40.0 (35.5 – 46.5)	0.195
VISA-A Interference Subscale	34.0 (25.0 – 40.0)	33.0 (25.0 – 37.0)	34.0 (29.0 – 40.0)	0.539
PPT A	432 (334 – 561)	423 (387 – 515)	408 (271 – 477)	0.455
PPT B	491 (378 – 588)	480 (384 – 561)	432 (319 – 518)	0.217
CPM	58.0 (21.0 – 107)	28.5 (-11.0 – 67.5)	36.0 (9.0 – 67.0)	0.121

Height is expressed as average ± standard deviation, while sex, dominant limb and main sport are represented as a percentage. The remaining variables are expressed as median (IQR).
† Ambidextrous participants: 11.5% (n=3), 8.8% (n=5), 6.3% (n=2) were *TT*, *CT* and *CC* genotypes, respectively.
‡ Other sports that the participants took part in: TT- 3.9% (n=1) cricket/golf; 11.5% (n=3) other, CT – 3.6% (n=2) cricket/golf; 3.6% (n=2) cycling; 3.6% (n=2) gym; 1.8% (n=1) triathlon; 8.9% (n=4) other; CC- 9.7% (n=3) gym; 6.5% (n=2) triathlon; 6.5% (n=2) other.
PPI – present pain intensity; VAS – visual analogue scale; CPM = PPT B – PPT A

Table 5.5: *TACR1* rs3771829 (C/G) genotype distributions for all the participants with chronic Achilles tendinopathy (AT) (i) demographic and injury characteristics, (ii) sporting history and training duration during the preceding 24 months, (iii) self-reported tendon pain from the short form McGill Pain Questionnaire (sf-MPQ), short form Brief Pain Inventory (sf-BPI) and the Victorian Institute Sports Assessment – Achilles (VISA-A), (iv) pressure pain thresholds (PPT) before (A) and during (B) ice emersion, and (v) conditioned pain modulation (CPM).

	CC (n=97)	**CG** (n=26)	**P-value**
Age at recruitment (years)	44.8 ± 10.8	43.6 ± 10.5	0.631
Sex (% male)	59.8	73.1	0.258
Height (cm)	172.4 ± 9.0	175.5 ± 9.1	0.146
Weight (kg)	75.6 ± 14.0	75.2 ± 13.4	0.873
BMI ($kg.m^{-2}$)	24.8 (23.0 – 27.8)	24.2 (21.7 – 27.4)	0.483
Dominant limb (% right)†	75.8	83.3	0.636
Bilateral AT (%)	35.5	19.2	0.103
Unilateral AT (% right)	38.7	34.6	0.103
Duration of symptoms (months)	12.0 (6.0 – 39.0)	10.0 (3.0 – 19.0)	**0.019**
Main Sport (% running)‡	84.1	76.0	0.321
Participation in main sport (yrs)	10.0 (5.0 – 20.0)	8.0 (4.0 – 20.0)	0.732
Training 1 to 3 months (hrs/wk)	6.0 (3.0 – 7.0)	5.5 (4.0 – 8.0)	0.386
Training 4 to 12 months (hrs/wk)	6.0 (4.0 – 7.5)	6.0 (5.0 – 8.0)	0.332
Training 13 to 24 months(hrs/wk)	5.0 (3.0 – 7.0)	7.0 (3.0 – 8.0)	0.214
sf-MPQ Total	7.3 (4.0 – 12.0)	8.7 (4.0 – 12.0)	0.566
PPI	1.0 (0.0 – 2.0)	1.0 (0.0 – 2.0)	0.696
VAS	0.6 (0.0 – 3.0)	0.6 (0.0 – 3.0)	0.896
sf-MPQ Sensory Subscale	3.0 (2.0 – 6.0)	4.0 (2.0 – 8.0)	0.364
sf-MPQ Affective Subscale	0.0 (0.0 – 0.0)	0.0 (0.0 – 1.0)	0.783
sf-BPI Total	16.0 (9.0 – 29.0)	15.5 (11.0 – 24.0)	0.959
sf-BPI Intensity Subscale	10.0 (6.0 – 14.0)	9.5 (7.0 – 15.0)	0.503
sf-BPI Interference Subscale	6.0 (1.0 – 17.0)	5.0 (2.0 – 13.0)	0.691
VISA-A Total	68.7 ± 16.6	74.8 ± 12.2	0.084
VISA-A Sensory Subscale	39.0 (33.0 – 45.0)	40.5 (33.0 – 47.0)	0.311
VISA-A Interference Subscale	33.0 (25.0 – 39.0)	34.0 (30.0 – 40.0)	0.222
PPT A	414 (345 – 491)	442 (388 – 570)	0.178
PPT B	519 (382 – 597)	453 (350 – 539)	0.163
CPM	34.0 (-2.0 – 77.0)	49.0 (3.0 – 85.0)	0.576

Height and total VISA-A score are expressed as average ± standard deviation, while sex, dominant limb and main sport are represented as a percentage. The remaining variables are expressed as median (IQR).
† Ambidextrous participants: 8.8% (n=8), 8.3% (n=2) were CC and *CG* genotypes, respectively.
‡ Other sports that the participants took part in: *CC*- 3.4% (n=3) cricket/golf; 2.3% (n=2) cycling; 3.4% (n=3) gym; 2.3% (n=2) triathlon; 4.6% (n=4) other, CG – 4.0% (n=1) gym; 4.0% (n=1) triathlon; 16.0% (n=4) other.
PPI – present pain intensity; VAS – visual analogue scale; CPM = PPT B minus PPT A

Table 5.6: *SCN9A* rs6746030 (G/A) genotype distributions for all the participants with chronic Achilles tendinopathy (AT) (i) demographic and injury characteristics, (ii) sporting history and training duration during the preceding 24 months, (iii) self-reported tendon pain from the short form McGill Pain Questionnaire (sf-MPQ), short form Brief Pain Inventory (sf-BPI) and the Victorian Institute Sports Assessment – Achilles (VISA-A), (iv) pressure pain thresholds (PPT) before (A) and during (B) ice immersion, and (v) conditioned pain modulation (CPM).

	GG (n=92)	**GA + AA** (n=23)[#]	**p-value**
Age at recruitment (years)	44.1 ± 10.6	45.0 ± 11.2	0.706
Sex (% male)	64.9	52.0	0.254
Height (cm)	173.4 ± 9.0	170.7 ± 7.8	0.445
Weight (kg)	75.9 ± 13.6	74.6 ± 14.1	0.792
BMI ($kg.m^{-2}$)	24.6 (22.8-28.0)	26.0 (24.1–27.4)	0.442
Dominant limb (% right)[†]	75.6	76.2	0.987
Bilateral AT (%)	29.7	37.5	0.501
Unilateral AT (% right)	37.4	41.7	0.501
Duration of symptoms (months)	12.0 (5.0 – 33.5)	12 (8.0 – 36.0)	0.340
Main Sport (% running)[‡]	80.7	81.0	0.096
Participation in main sport (yrs)	8.5 (5.0 – 20.0)	11.0 (4.0 – 25.0)	0.970
Training 1 to 3 months (hrs/wk)	5.0 (3.0 – 7.0)	5.0 (2.5 – 7.0)	0.736
Training 4 to 12 months (hrs/wk)	6.0 (4.0 – 8.0)	5.5 (2.3 – 7.0)	0.294
Training 13 to 24 months (hrs/wk)	6.0 (3.0 – 8.0)	5.0 (2.0 – 8.0)	0.621
sf-MPQ Total	7.9 (4.0 – 12.0)	6.5 (3.0 – 14.8)	0.846
PPI	1.0 (0.0 – 2.0)	1.0 (0.0 – 2.0)	0.731
VAS	1.2 (0.0 – 3.5)	0.0 (0.0 – 3.3)	0.494
sf-MPQ Sensory Subscale	3.0 (2.0 – 6.0)	3.0 (1.5 – 8.5)	0.981
sf-MPQ Affective Subscale	0.0 (0.0 – 0.5)	0.0 (0.0 – 0.5)	1.000
sf-BPI Total	16.0 (9.0 – 29.0)	22.5 (11.0 – 29.0)	0.489
sf-BPI Intensity Subscale	10.0 (6.0 – 14.0)	11.0 (7.0 – 15.0)	0.473
sf-BPI Interference Subscale	6.0 (1.0 – 15.0)	11.5 (3.0 – 15.5)	0.325
VISA-A Total	70.7 ± 15.6	67.0 ± 17.4	0.317
VISA-A Sensory Subscale	39.0 (33.0 – 46.0)	38.0 (31.0 – 42.5)	0.517
VISA-A Interference Subscale	34.0 (27.0 – 40.0)	31.5 (25.0 – 36.0)	0.197
PPT A	419 (371 – 513)	388 (289 – 515)	0.282
PPT B	460 (362 – 562)	448 (323 – 539)	0.532
CPM	34.0 (-10.0 – 77.0)	34.0 (8.0 – 90.0)	0.893

Height and total VISA-A scores are expressed as average ± standard deviation, while sex, dominant limb and main sport are represented as a percentage. The remaining variables are expressed as median (IQR).
[#] GA: n=22 and AA n=1
[†] Ambidextrous participants: 8.9% (n=8), 9.5% (n=2) were *GG* and *AG + AA* genotypes, respectively.
[‡] Other sports that the participants took part in: GG- 3.4% (n=3) cricket/golf; 2.3% (n=2) cycling; 5.7% (n=5) gym; 3.4% (n=3) triathlon; 4.6% (n=4) other, *AA +AG* – 19.1% (n=4) other.
PPI – present pain intensity; VAS – visual analogue scale; CPM = PPT B minus PPT A

The median total scores of self-reported tendon pain from the sf-MPQ were significantly different (p=0.019) among the three *COMT* rs4818 (G/C) genotype groups [median (IQR)- CC: 9.1 (4.0 –13.0) n=61; CG: 7.3 (4.0 – 0.0) n=50; GG: 4.0 (1.0 – 5.0) n=7], with the CC genotype having a significantly higher pain score (p=0.018) than the GG genotype (Figure 5.1A). The median present pain intensity (PPI) scores were also significantly different (p=0.046) among the three genotype groups [median (IQR) - CC: 1.0 (0.0 – 2.0), n=61; CG: 1.0 (0.0 – 2.0), n=50; GG: 0.0, (0.0 – 1.0), n=7] (Figure 5.1B). However, after the post-hoc analysis only a trend (p=0.054) was noted for the CC genotype having a higher pain score than the GG genotype. No significant differences in the rs4818 genotype distributions were noted for the total VAS [median (IQR)-CC: 1.4 (0.0 – 3.9), n=61; CG: 0.6 (0.0 – 2.1), n=50; GG: 0.0 (0.0 – 1.0), n=7; p=0.158] (Figure 5.1C); the sf-MPQ sensory [median (IQR)-CC: 3.0 (2.0 – 7.0), n=61; CG: 3.5 (2.0 – 6.0), n=50; GG: 3.0 (1.0 – 4.0), n=7; p=0.461] (Figure 5.1D) and affective [median (IQR)- CC: 0.0 (0.0 – 1.0), n=61; CG: 0.0 (0.0 – 0.0), n=50; GG: 0.0 (0.0 – 0.0), n=7; p=0.226] (Figure 5.1E) subscale scores.

When divided into the lower (scores ranging from 0 to 7.5) and upper (scores ranging from 7.8 to 57) total sf-MPQ scores, the relative frequency of the *COMT* rs4818 GG genotype was significantly over-represented (p=0.013) in the lower score group (Figure 5.2A). Similarly, the relative frequency of the GG genotype was significantly over-represented in the lower score group of the PPI (lower score range 0 to 1, upper score range 2 to 5, p=0.040) (Figure 5.2B), the VAS (lower score range 0 to 1.1, upper score range 1.3 to 10, p=0.015) (Figure 5.2C), the sf-MPQ sensory (lower score range 0 to 4, upper score range 5 to 33, p=0.042) (Figure 5.2D), but not the sf-MPQ affective (lower score 0, upper score range 1 to 9, p=0.194) (Figure 5.2E) subscales. Although

not significant, it is interesting to note that similar to the other subscales, no GG genotypes were identified in the upper score group of the sf-MPQ affective subscale.

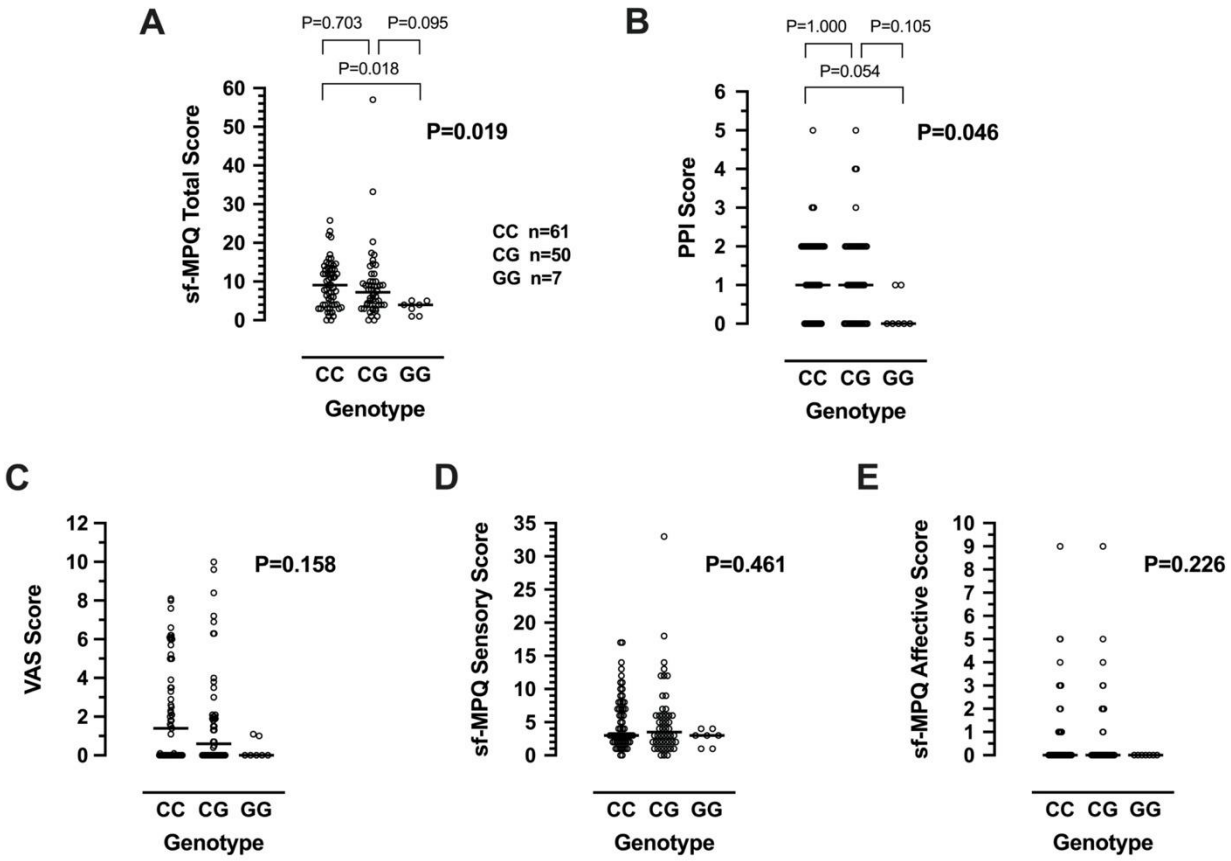

Figure 5.1: Genotype distributions for the tendon pain scores for all participants with chronic Achilles tendinopathy evaluated using the short form McGill Pain Questionnaire (sf-MPQ). Scatterplots and medians of the scores are represented **(A)** of self-reported tendon pain from the total score of sf-MPQ, **(B)** present pain intensity (PPI), **(C)** visual analogue scale (VAS), **(D)** sf-MPQ Sensory subscale and **(E)** sf-MPQ Affective subscale of the three *COMT* rs4818 (G/C) genotype groups of all the participants with chronic Achilles tendinopathy. Global p-values are indicated in bold and the post-hoc analysis P-values are also indicated when the global p-value was significant.

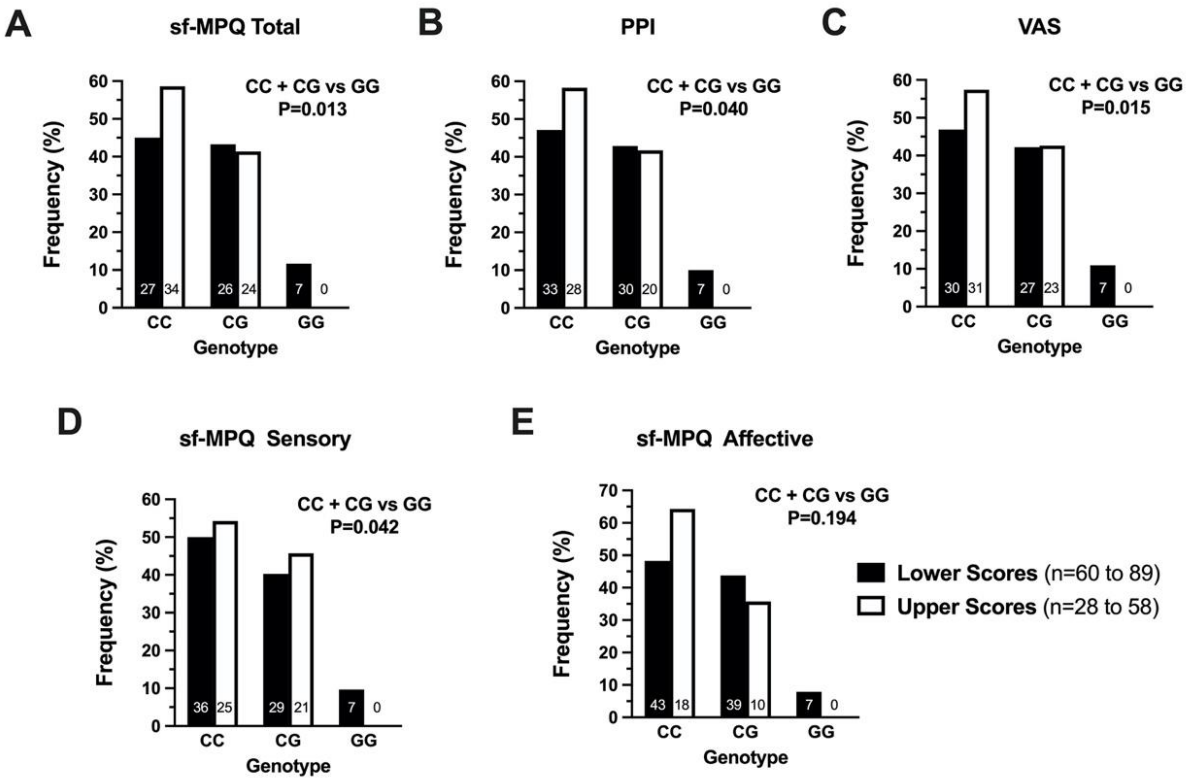

Figure 5.2: Genotype distributions of *COMT* rs4818 (G/C) in the lower (black bars) and upper (clear bars) scores of the **(A)** the total self-reported tendon pain scores from the short form McGill Pain Questionnaire (sf-MPQ), **(B)** the present pain intensity (PPI) score, **(C)** the total visual analogue score (VAS), **(D)** the total sensory subscale score of the sf-MPQ and **(E)** the total sf-MPQ affective subscale score of the sf-MPQ questionnaire in all the participants with chronic panful Achilles tendinopathy. The n values of the lower and upper scores for each genotype is embedded in the bars and total n value ranges of the lower and upper score groups is indicated in the figure legend. The p values are also given. The lower scores ranged from 0 to 7.5; 0 to 1; 0 to 1.1 and 0 to 4 for the total sf-MPQ, PPI, VAS and sf-MPQ sensory subscale, respectively. The lower score of sf-MPQ affective subscale was zero. The upper scores ranged from 7.8 to 57, 2 to 5; 1.3 to 10; 5 to 33 and 1 to 9 for the total sf-MPQ, PPI, VAS, the sf-MPQ sensory and the sf-MPQ affective, respectively. It was not possible to divide the scores into upper and lower halves for some of the scales because of the large number of small values, such as zero, which were all grouped together as low scores.

When *COMT* rs4633, *TAC1* rs2072100, *TACR1* rs3771829 and *SCN9A* rs746030 were analysed, there were no significant differences between the genotype distributions and the median self-reported total tendon pain scores for the sf-MPQ or the subscales (Table 5.2). Similarly, when divided into the lower and upper total sf-MPQ score halves, no significant differences in the genotype distributions were noted between the two groups (Figure 5.3).

No significant differences in the genotype distributions were noted for any of the five polymorphisms investigated when the self-reported total tendon pain scores from the total sf-BPI, the sf-BPI intensity and interference subscales were evaluated, (Tables 5.2- 5.6). Similarly, when divided into the lower (scores ranging from 0 to 15) and upper (scores ranging from 16 to 101) total sf-BPI score halves, there were no significant differences in the genotype distributions between the two groups (Figure 5.4).

Similarly, no significant differences in the genotype distributions were noted for any of the five polymorphisms investigated when the VISA-A scores, as well as the scores for the VISA-A sensory and interference subscales were evaluated (Tables 5.2-5.6). Moreover, when divided into the lower (scores ranging from 27 to 71) and upper (scores ranging from 72 to 100) total VISA-A score halves, no significant differences in genotype distributions between the two groups (Figure 5.5) were noted for any of the five polymorphisms.

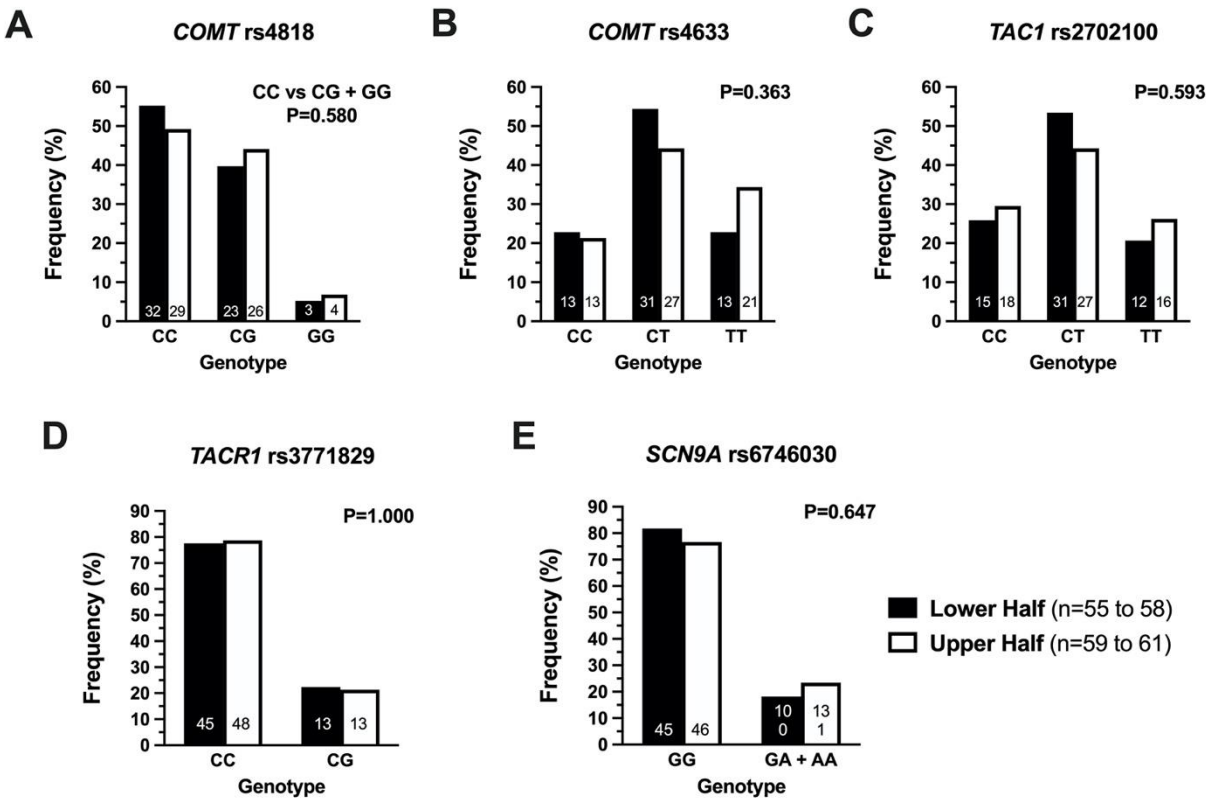

Figure 5.3: Frequency distributions of the **(A)** *COMT* rs4818 (G/C) **(B)** *COMT* rs4633 (C/T) **(C)** *TAC1* rs2072100 (C/T) **(D)** *TACR1* 3771829 (C/G) **(E)** *SCN9A* rs6746030 (G/A) genotypes in the lower (scores ranging from 0 to 15; black bars) and upper halves (scores ranging from 16 to 101; clear bars) of the total self-reported tendon pain scores from the short form Brief Pain Inventory (sf-BPI) of all the participants with chronic panful Achilles tendinopathy. The n values of the lower and upper halves for each genotype is embedded in the bars and total n value ranges of each half is indicated in the figure legend. The p values are also given. Differences in the lower and upper halve n values are due to same scores been group into the same half and/or missing genotype data. The n values for *SCN9A* rs6746030 are combined with the lower n values (0 and 1) representing the AA genotype in the respective groups.

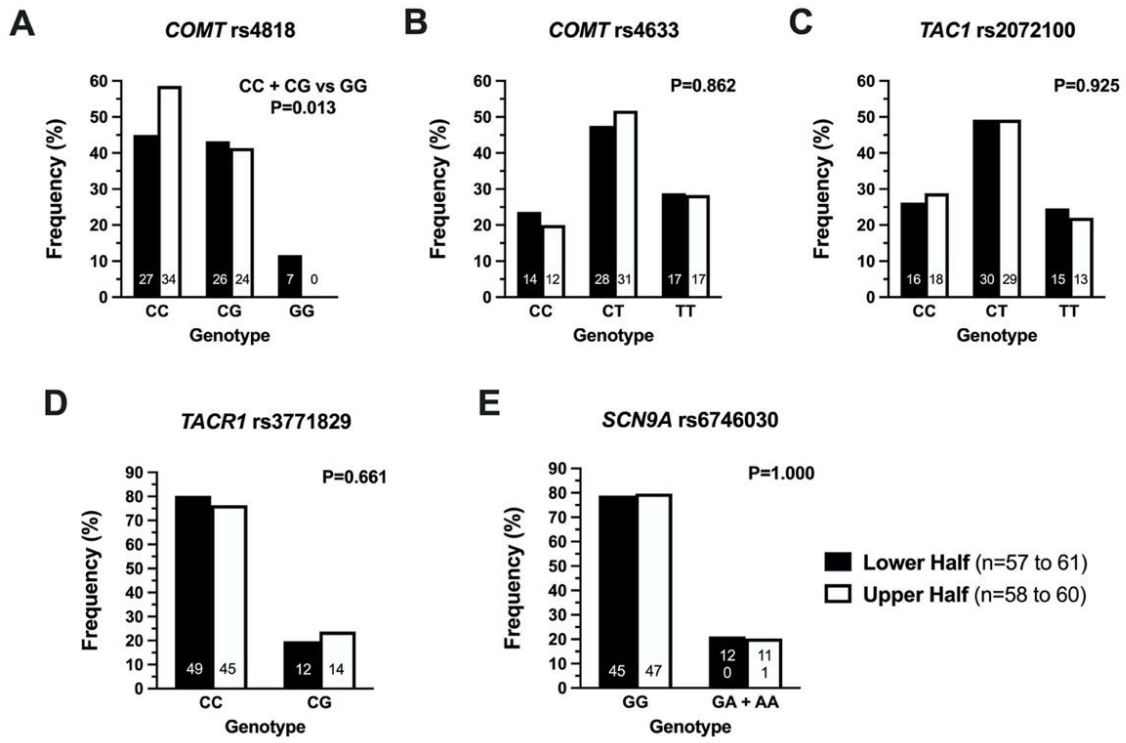

Figure 5.4: Frequency distributions of the **(A)** *COMT* rs4818 (G/C) **(B)** *COMT* rs4633 (C/T) **(C)** *TAC1* rs2072100 (C/T) **(D)** *TACR1* 3771829 (C/G) **(E)** *SCN9A* rs6746030 (G/A) genotypes in the lower (scores ranging from 0 to 7.5; black bars) and upper halves (scores ranging from 7.8 to 57; clear bars) of the total self-reported tendon pain scores from the short form McGill Pain Questionnaire (sf-MPQ) of all the participants with chronic panful Achilles tendinopathy. The n values of the lower and upper halves for each genotype is embedded in the bars and total n value ranges of each half is indicated in the figure legend. The p values are also given. The n values for *SCN9A* rs6746030 are combined with the lower n values (0 and 1) representing the AA genotype in the respective groups.

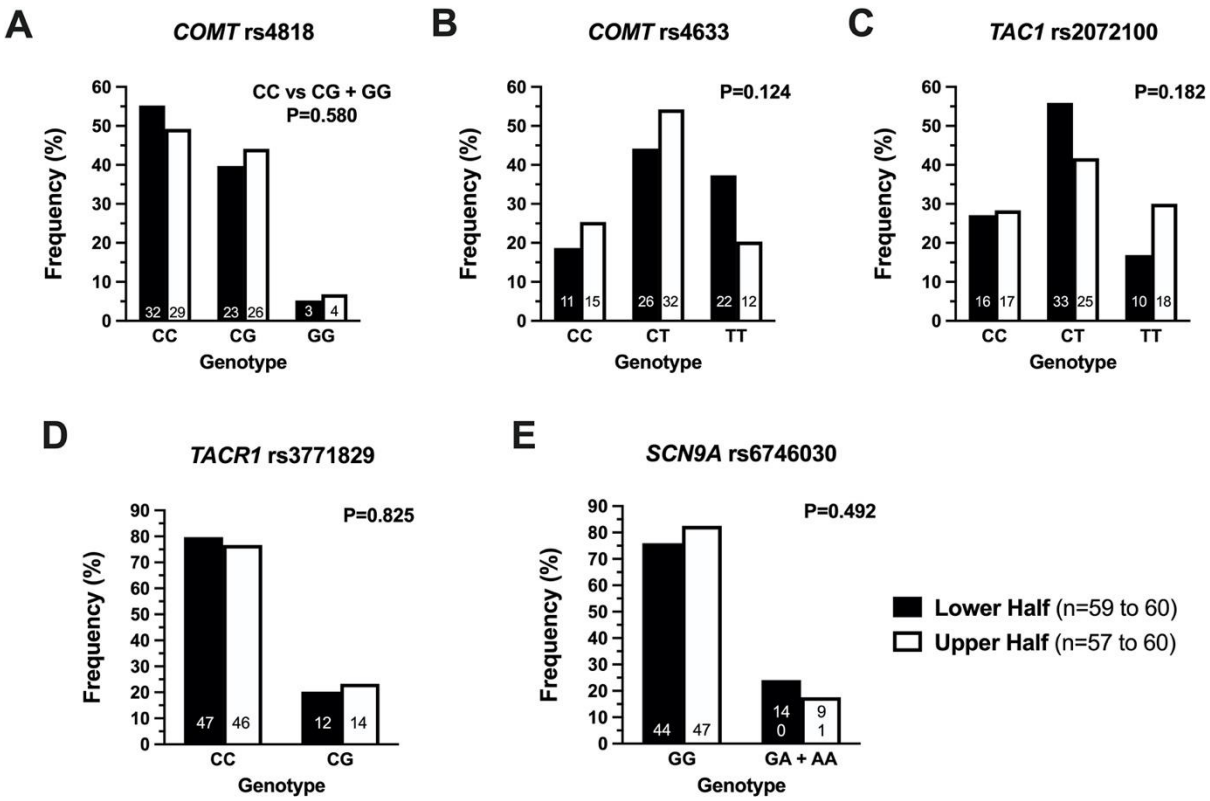

Figure 5.5: Frequency distributions of the **(A)** *COMT* rs4818 (G/C) **(B)** *COMT* rs4633 (C/T) **(C)** *TAC1* rs2072100 (C/T) **(D)** *TACR1* 3771829 (C/G) **(E)** *SCN9A* rs6746030 (G/A) genotypes in the lower (scores ranging from 27 to 71; black bars) and upper halves (scores ranging from 72 to 100; clear bars) of the total self-reported Victorian Institute Sports Assessment - Achilles (VISA-A) of all the participants with chronic panful Achilles tendinopathy. The n values of the lower and upper halves for each genotype is embedded in the bars and total n value ranges of each half is indicated in the figure legend. The p values are also given. Differences in the lower and upper halve n values are due to same scores been group into the same half and/or missing genotype data. The n values for *SCN9A* rs6746030 are combined with the lower n values (0 and 1) representing the AA genotype in the respective groups.

Finally, there were no significant differences between PPT before and during ice immersion, as well as the CPM between any of the different genotypes of the five polymorphisms (Tables 5.2-5.6). Similarly, when divided into the lower (scores ranging from -331 to 34) and upper (scores ranging from 36 to 547) CPM score halves, no significant differences in the genotype distributions between the two groups (Figure 5.6) were noted.

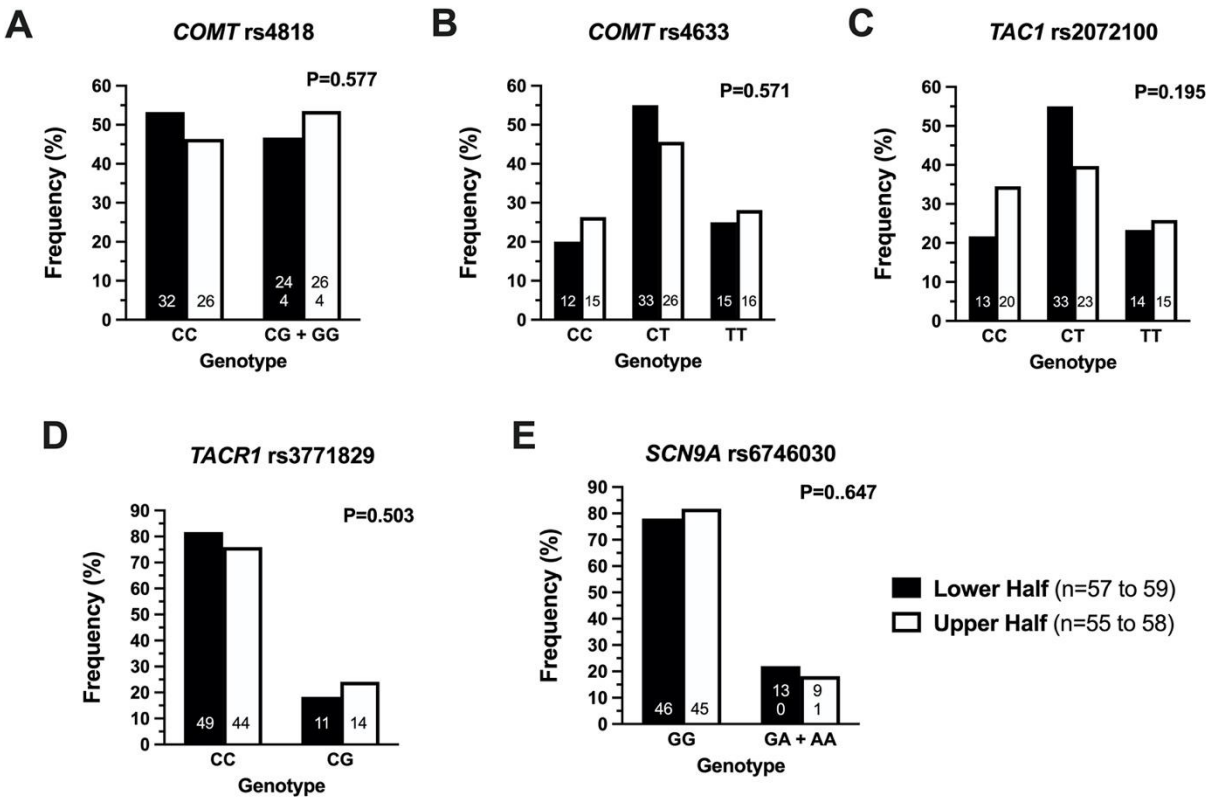

Figure 5.6: Frequency distributions of the **(A)** *COMT* rs4818 (G/C) **(B)** *COMT* rs4633 (C/T) **(C)** *TAC1* rs2072100 (C/T) **(D)** *TACR1* 3771829 (C/G) **(E)** *SCN9A* rs6746030 (G/A) genotypes in the lower (scores ranging from -331 to 34; black bars) and upper halves (scores ranging from 36 to 547; clear bars) of the conditioned pain modulation of all the participants with chronic panful Achilles tendinopathy. The n values of the lower and upper halves for each genotype is embedded in the bars and total n value ranges of each half is indicated in the figure legend. The p values are also given. Differences in the lower and upper halve n values are due to same scores been group into the same half and/or missing genotype data. The n values for *SCN9A* rs6746030 are combined with the lower n values (0 and 1) representing the AA genotype in the respective groups.

5.4. DISCUSSION

The novel finding of this chapter was that the functional variant within *COMT* was associated with tendon pain intensity in a cohort of recreational South African athletes with chronic Achilles tendinopathy. Specifically, the *COMT* rs4818 CC genotype was associated with higher total sf-MPQ scores and higher PPI. Additionally, the GG genotype was overrepresented in the lower half of grouped total, PPI, sensory index and visual analogue scale scores of the sf-MPQ illustrating an association with lower levels of pain perception. This polymorphism was however, not associated with the sf-BPI or VISA-A scales or sub-scales, as well as pressure pain thresholds and CPM. Moreover, none of the other four polymorphisms investigated were associated with the pain scores, pressure pain thresholds or CPM. This is the first study to the author's knowledge to examine the association of common genetic variants in the pain pathway with chronic pain in tendinopathy.

Previous investigators have reported that *COMT* rs4818 is associated with pain in several cohorts[442,605,606]. Similar to this study, other authors have previously reported an association of the *COMT* rs4818 C allele or the CC genotype with pain[442,443] and that high pain haplotypes contain the C allele[605]. However, other work has shown that this variant is not associated with PD related pain[440], experimental pain[441], lumbar radicular pain[46], recovery from whiplash[458] or migraine[607]. Perhaps ethnic differences could explain the changes in association in the various cohorts.

Although the *COMT* rs4818 polymorphism is synonymous, it has been reported to account for a greater variation in COMT enzyme activity compared to the more

routinely investigated functional Val158Met rs4680 polymorphism[464]. One possible explanation for the enhanced pain scores with the rs4818 C-allele could therefore be linked to decreased enzymatic activity of COMT[605]. The converse for the G-allele may be true, which aligns with this chapter's associations. Additionally, *COMT* variants have been associated with modulation of depressive symptoms and maladaptive coping mechanisms[352,440,441]. The latter are associated with pain intensity and disability, which could help explain the increased pain scores in this cohort as tendon pain has been shown to have a significant psychological component[100]. Although the second *COMT* synonymous polymorphism investigated in this study, rs4633, has been associated with LBP related disability[457], no associations were reported with pain scores, pressure pain thresholds or CPM in this study. Future work however should investigate other *COMT* polymorphisms, including the functional Val158Met rs4680 polymorphism, with pain sensitivity and self-reported pain associated with chronic Achilles and other tendinopathies.

Previous research on *TACR1* variants has focused on psychological and psychiatric conditions and the role of *TACR1* in pain has been less explored. *TACR1* encodes the receptor NK-1R and the role in pain of NK-1R's main ligand, SP, has been explored widely. In the present study, no associations were observed between *TACR1* rs3771829 (C/G) and pain scores, pressure pain thresholds or CPM. Similarly, previous work also found no associations between *TACR1* polymorphisms and pain in a fibromyalgia cohort[608], in women with endometriosis[609] or in individuals with cancer induced bone pain[610]. In contrast, *TACR1* rs11688000 was associated with decreased pain in knee OA[611]. While these findings may be discouraging, other evidence make *TACR1* an attractive candidate for study in chronic tendon pain. Murine

models have shown that NK-1R mediates hyperalgesia[612] and that the wind-up effect of pain is NK-IR dependent[612]. Both hyperalgesia and wind-up have been discussed in relation to pain in tendinopathy[87], therefore NK-1R, and by extension *TACR1*, may also be relevant to pain responses in tendinopathy. Since rs11688000 is intragenic, its function is unknown. However, it could influence transcription and hence gene function. Therefore, more work on this gene and its variants is required in order to further explore its biological relevance to tendon pain mechanisms.

No associations were observed between *SCN9A* rs6746030 (G/A) and self-reported pain, PPT or CPM in this cohort. Similarly, Cassao et al.[613] did not observe associations between rs6746030 and painful bladder syndrome despite observing it with other polymorphisms. This, however, contrasts with previous studies that have shown an association with lumbar root pain, musculoskeletal Parkinson's disease-related pain and painful bladder syndrome[399,440,602,614]. Individuals with the minor A allele had a greater pain experience in these studies. The A allele of this gene results in an amino acid substitution from arginine (R) to tryptophan (W) at position 1150 of the encoded sodium channel $Na_v1.7$[398]. Functional work has demonstrated that Nav1.7–1150W (encoded by the minor A allele) depolarises the resting membrane potential and doubles the firing frequency in response to depolarisation in DRG neurons, which affects their sensitivity to painful stimuli[398]. Additionally, in a subset of healthy individuals, the A allele of rs6746030 was associated with decreased pain thresholds for various noxious stimuli especially those that evoked predominantly C-fibre mediated pain[399]. This implies that C fibre activation, which underlies dull, aching pain, such as tendon pain **(Chapter 2)** represents the nociceptive pathway mediating A allele driven clinical pain and hence the importance of *SCN9A* rs6746030 in the

study of tendon pain.

Larger cohorts should in future explore haplotype block analysis, which involves the evaluation of several polymorphisms as opposed to single polymorphisms and thereby potentially capturing more information on the relationships between these gene variants and the observed phenotype. The differences noted in the literature could potentially be explained by sample heterogeneity: the conditions studied are varied and or the confounding effects from potentially comparing different aetio-pathologies.

In conclusion, the causes of pain in chronic AT are not yet fully described amid vast inter-individual differences. Recent work in other painful conditions has shown that genetic variation likely accounts for some of these differences in chronic pain profiles. As the cause and mechanisms of tendon pain are unknown, identifying genes that may be involved could help explain the pain mechanisms in chronic AT. In this chapter, *COMT* rs4818 was associated with self-reported tendon pain, suggesting that the catecholaminergic pathway is involved in the aetiology of chronic pain in AT. However, more work should be conducted on larger sample sizes and on more gene panels in order to broaden our understanding of pain genetics in tendinopathy.

CHAPTER SIX

6.1. SUMMARY AND PERSPECTIVES

AT is a common injury, which not only affects athletes in running sports but also affects sedentary individuals[2–5,191]. Tendon pathology can exist without pain[7]; however, in most cases, pain is the hallmark of the clinical condition[85,507]. Achilles tendon pain, like any other tendon pain, is often of insidious onset with no definitive inciting event[550]. It is resistant to available treatments and often becomes chronic[32]. As reviewed in **Chapter 1**, a few hypotheses have been put forward to explain the potential causes or mechanisms of tendon pain. The main theories postulate that tendon pain is a result of inflammation in the tendon[33,34], or matrix degeneration[35], production of neuropeptides and other metabolites[37,38] or changes in central pain mechanisms[39]. Additionally, evidence from other musculoskeletal pain conditions provides a strong theoretical framework for the role of genetic variation in tendon pain[44–47]. Understanding the sources and/or mechanisms of tendon pain is important in order to develop more efficacious treatments for tendinopathy.

The aims of this thesis were to characterise Achilles tendon pain using other pain questionnaires, to investigate the relationship between structural changes and central pain mechanisms with self- reported tendon pain. Additionally, the thesis sought to evaluate the relationship between selected gene variants and pain in a cohort of recreational athletes with chronic Achilles tendinopathy using a candidate gene approach. In **Chapter 2** of the thesis, a novel assessment of AT pain was conducted where individuals described their tendon pain symptoms using the traditional VISA-A as well as two questionnaires, namely the sf-MPQ and sf-BPI, not previously used to

assess AT pain. These questionnaires were selected for their ease of administration and the type of details that they would provide. They were originally developed for cancer pain but have since been validated in other pain syndromes[484–486,615,616]. The sf-MPQ in particular allows for the multidimensional interrogation of the pain experience using various adjectives to describe pain[615]. In this cohort, Achilles tendon pain was generally described as 'aching' (60.3%), 'tender' (52.9%) and 'throbbing' (33.9%) **(Chapter 2).** These are word descriptors for nociceptive/inflammatory pain. In particular, 'aching' is a common descriptor of musculoskeletal pain[617]. The language of pain was previously shown to be condition specific with sufferers often using particular sets of words to describe similar pain syndromes[618]. The importance of using multidimensional pain scales such as sf-MPQ to describe tendon pain is the added benefit that they also capture the perceptual qualities of pain, which would otherwise not be reported on a numerical pain rating scale (NPRS)[619]. For example, hot-burning and sharp pain in the arm is different from a dull ache, even though both may be scored as 7/10 on NPRS and be equally debilitating with the conduct of activities of daily living (ADLs). The treatment strategies for these two types of pain would also be different. To date, the assessment of the perceptual qualities of tendon pain has not been widely reported in the literature, which may highlight, in part, our limited understanding of it.

Word descriptors for pain are crucial in arriving at a diagnosis as well as choice of treatment[618,619]. This ability to describe pain using various word choices allows for the monitoring of changes in pain quality and its severity. As reflected in studies, which have shown that as treatment or pathology progresses, the adjectives used to describe pain on the sf-MPQ also change, which can be monitored during rehabilitation[620]. Previous work has also shown that the number of words chosen on the sf-MPQ are

also reflective of pain severity especially of a sensory kind[503]. For this reason, we were particularly interested in capturing the most used descriptors in the sensory index, to better characterise their relationship with tendon pain. This meant that words that were not used commonly in the sensory (cramping, gnawing, heavy and splitting) and affective (tiring-exhausting, fearful, punishing-cruel and sickening) indices were not further analysed in this thesis. In the sensory index, the less commonly used terms denote continuous pain (cramping, gnawing and heavy) and intermittent pain (splitting). However, the most used terms in this cohort also denoted continuous and intermittent pain **(Chapter 2).** Therefore, while there seems to be no apparent trend in this regard, future studies should also explore these words that are not commonly used in order to improve our understanding of tendon pain as described on the sf-MPQ and the implications of these word choices in the clinical presentation of tendon pain. A much larger sample size would however be required to analyse these less commonly used words.

Chapter 2 also established that far from being only a physical condition, AT also has effects on relations with other people, sleep, and mood. This observation was interesting as minimal affective components were observed. Recent qualitative work has explored the psychosocial impact of tendon pain and shown that in addition to affecting running and ADLs, tendon pain also has a significant psychological burden especially in those individuals who attach a high worth to their physical capabilities[99,132,621]. The collective findings therefore suggest that perhaps tendon pain ought to be assessed, managed and treated like any other type of chronic pain. With an equal emphasis on the pain quality, its descriptors and its interferences that are beyond sporting function such as sleep disturbance. This is not a novel concept, as it

is in general, advocated for as best practice for other musculoskeletal and chronic pain syndromes[622–626]. Perhaps, following a more holistic approach, using the biopsychosocial model of assessment and treatment,, clinicians and researchers would improve the understanding of tendon pain and the spectrum of its consequences allowing for improved care and an outcome where patients would feel less frustrated about their symptoms[621,622,627].

Additionally, while tendon pain did affect sleep, mood and relations with other people, as would be expected for any chronic condition, the effect was minimal despite the long duration of symptoms noted in this cohort. This may be a function of personality and its association with the pain experience[628–631]. AT is common in runners and in this cohort, the runners were training for an ultramarathon event and continued to do so in spite of their pain. Therefore, perhaps the enigma of Achilles tendon pain mechanisms also lies in *who* gets the pain. The kind of individual who trains for and races in ultramarathons is one who has great mental and physical fortitude and can persevere despite frequent and prolonged exposure to exercise induced painful stimuli[632]. Given the nature of endurance sport, and also as a result of it, the individuals who choose it as a recreational activity and consequently develop AT are presumably tough as nails, which explains their trivialisation of AT pain as an annoyance[586,632,633]. This suggests an association between chronic Achilles tendon pain experience and personality as demonstrated in other conditions[519–522,590,591].

Personality traits such as higher self- efficacy[629,634], pain catastrophising[132,516–518,521,634–637], neuroticism[638], impulsivity and anxiety[639], borderline personality disorder[640], novelty seeking and harm avoidance personalities[641] have been shown to modulate the pain response in a variety of pain disorders. Additionally, a handful of

studies on psychological factors associated with assorted tendinopathies have also been conducted though the results are thus far conflicting[132,621,642]. From these and other studies, it is therefore not unreasonable to presume then that a similar relationship may exist between Achilles tendon pain and personality. This is an area that warrants further investigation.

Chapter 3 of the thesis explored the tendon structural changes as a potential source of pain. Altered tendon structure has been implicated in the pain pathway of tendinopathy in some studies[257,277,643] but not in others[13,254,289–291,644], the present study included. Hence, the association between tendon structure and pain remains equivocal. However, US findings may have some clinical utility in predicting future tendinopathy[258,280] or as a prognostic marker[35]. A novel aspect of this thesis was the use of various multidimensional pain scales to assess pain. While pain descriptors are associated with structure in other disease cohorts[618], this was not generally the case in this cohort. This loose and inconsistent association between structural findings and pain further illustrates the need to identify the type of pain prior to treatment and reinforces the need to capture the multidimensional nature of pain to aid in this identification process[645]. After all, treatment strategies are more impactful when individual mechanisms are targeted with specific solutions[646]. To that end, a notable finding from this study was that while US abnormalities were not associated with pain, they were associated with higher interference index scores of the VISA-A as well as interference scores for general activity and walking ability on the sf-BPI. Which perhaps suggests that using pain intensity as a primary outcome measure of rehabilitation or treatment efficacy may be insufficient[257,267,293,647]. This limitation as an outcome measure is all the more important because tendon tissue has a very low

turnover[84], while the pain system has high plasticity[648] so changes in the pain profile may occur long before the structure is restored. As a result, perhaps the focus ought to be more towards the relationship between function and structure rather than with pain in isolation. After all, studies have shown that functional measures such as tendon mechanical properties, namely tendon strain, shear modulus and viscosity, are impaired in tendinopathy, which negatively affects the tendon's ability to efficiently store and release elastic energy during locomotion and explosive activities[647].

In clinical practice, tendon structure is visualised using US/CD and MRI. However, these technologies come with a number of limitations such as inter-observer differences and variations in protocols across different institutions[305]. In response, some authors have advocated for standardisation of the US method of assessment to minimise measurement variations[649–651] and other techniques such as CEUS and UTC have been developed and utilised to provide some objectivity in the analysis of tendon structure[295,546,548,549]. There is still however, an urgent need to standardise current technologies or develop more objective ones to improve their utility in clinical practice and research.

Chapter 4 of this thesis explored altered CPM as another potential source of pain in tendinopathy. It is generally accepted that some functional changes in the brain occur in response to chronic pain[652] and CPM is one method of assessing these functional changes[653,654]. In this cohort, individuals with tendinopathy did not differ in central pain mechanisms when compared to pain-free controls. These results are in stark contrast with the only other paper on this subject, which showed a lowered CPM in Achilles tendinopathy[39] but are consistent with the results of **Chapter 2,** which showed a

minimal affective component in Achilles tendinopathy. Regardless of the current contradictions in the literature, that central mechanisms are likely involved in AT is seen in that CPM is altered in other lower limb conditions. For example, altered CPM is observed in chronic patellofemoral pain[553,557] and gluteal tendinopathy[655]. Additional evidence for the role of central mechanisms in AT is shown in that bilateral sensitisation and US abnormalities are observed in unilateral symptomatic tendinopathy[133,656] and lower PPT and generalised hyperalgesia observed in chronic AT[657]. Recent evidence further suggests the involvement of central mechanisms in tendinopathy as reflected in individuals with asymptomatic tendinopathies in whom PPT were higher than normal, which suggests central *desensitisation* as a reason for remaining pain free despite imaging evidence of pathology[570]. Furthermore, the most effective treatment for AT to date is exercise[550,551,576], whose efficacy is thought to be centrally mediated[568]. However, much work on CPM in tendinopathy has been conducted in the upper limb tendons and it is generally accepted that CPM is lowered following upper limb tendinopathy[40,335] though not consistently[571]. Hence, altered central pain mechanisms may explain upper limb tendon pain. In AT though, the present is only the second study, which makes direct comparisons rather difficult. While there are several studies on CPM in upper limb tendinopathies with which to compare, the differences in upper limb and lower limb tendon structure and hence function[71,658], make these comparisons rather tenuous at best.

Studies on CPM have also used different paradigms (time parameters, type of stimulus and method of stimulus presentation) resulting in a spectrum of conclusions[566,659,660]. Some cohorts have used highly trained athletes where others have used physically active people; intense endurance training has been shown to affect CPM compared to

regular exercise activity[508,586,633,661,662]. In addition, the type of stimulus is varied; cold[555,565] heat[663] or pressure[553,557]; different conditioning stimuli can lead to different intensities of CPM even in the same individual[664]. Where the stimulus is the same, such as cold, the temperature is different[555,565,566,586] or the point of application of the stimulus is different[39,313,554]. Hence, reliability of CPM results cannot be consistently extrapolated from one study to the other[665]. In cohorts where they may have used more than one observer, inter-observer reliability could be a problem[565]. Additionally, some studies have showed that though CPM may be less efficient in chronic pain states, it can 'normalise' with the chronicity of the syndrome[666] perhaps through repeated exposure to pain, leading to adaptation in the nucleus accumbens-medial prefrontal cortex functional connectivity and in sensorimotor network connectivity with the brain cortices, which are involved in pain modulation[667]. This may also explain the conflicting results on CPM in the literature[668]. Also to be considered is the heterogeneity of conditions, as even in the same syndrome such as chronic LBP, a spectrum of results has been observed in central sensitisation[669]. Another consideration to be made is the effect of psychological factors such as personality, anxiety and depression on the CPM effect[670]. These are seldom studied as a primary outcome even though their effect on the pain experience is known and psychological factors are prognostic influences on the outcomes of rehabilitation[627,671,672]. Experts on CPM agree that this technique has a lot to offer in deciphering central pain mechanisms in chronic conditions. This is all the more important in clinical practice as CPM differences have been shown to affect response to analgesic treatment[673]. However, CPM experts also acknowledge that currently no paradigm or method of evoking the CPM is superior to the other[567]. While this validates work done on CPM to date, it still makes comparisons across studies complicated.

Chapter 5 of this thesis explored a hypothesis that postulates that genetic variations may, at least in part, be responsible for the variations in clinical presentation that are observed with tendon pain. In addition to being heavily influenced by the environment, genetic variations account for a significant component of the overall pain experience with heritability ranges between 22–60%[674]. While novel in tendinopathy, the notion of pain genetics has been extensively studied in other painful conditions[675–677]. In particular, *COMT*[455,674,678–681], ion channel genes[397,682,683], and cytokine related genes[424,684–687] are the most commonly studied pain pathway genes while fibromyalgia[454], migraine, diabetic neuropathy[677], LBP[46] and chronic post-surgical pain[413,687] are among the most widely studied pain conditions. Hence, the choice of genes in this study was based on the biological importance of their encoded proteins in the pain pathway (COMT, $Na_v1.7$) and previous associations of the proteins in tendinopathy (SP and its receptor). The present study found that individuals who carried the CC genotype of *COMT* rs4818 (C/G) reported higher pain scores. This is consistent with similar studies, which have implicated the C allele in the high pain phenotypes[606,688]. The C allele leads to the production of lower concentrations of COMT, which results in increased pain report[606,688]. Conversely, the GG genotype was overrepresented in the low pain score groups, which suggests that it leads to production of more COMT and hence decreased pain report. Genetic variations have also been shown to affect treatment in the physiotherapy management of OA[595] and more commonly in response to opioid treatment[675,689–692]. On a background were genetics modulates risk[380] and severity of diseases[454], pain perception and analgesic use[452,693,694] and response to treatment[595], it may be worth exploring the genetics of tendon pain as it may well lead to a better understanding of the biological mechanisms

of this type of pain. Also, if Govil et al's study[595] is anything to go by, genetics may well explain why tendon pain is stubborn to treatment.

Additionally, it is possible that AT presents as various sub-types as in LBP and in these sub-types, patients describe their pain using particular adjectives as one would for a separate pathology. By this hypothesis, individuals' pain derives from different pathologies and therefore would need to be managed that way. For example, perhaps in some individuals, tendon degeneration is the predominate source of pain, while it may be inflammation, neovascularisation, neuropeptides or altered pain mechanisms in others. Perhaps in our pursuit to establish a *singular* source of tendon pain we have overlooked the possibility of a multiplicity of sources. This hypothesis could explain the variations in success rates of some AT pain treatments. If one has, hypothetically, a neovascular tendon pain, then they would respond well to sclerosing treatments while others would fail to replicate the same results. Additionally, for inflammatory pain, then anti-inflammatory treatments would be ideal. Ultimately though, who develops what subtype may be at least in part influenced genetically, which underscores the importance of considering genetics in the continual study of chronic pain.

This body of work is not without limitations. Firstly, pain is a complex symptom, which has gender, ethnic[695] and epigenetic drivers[424]. This thesis only focused on one aspect of this pain and would benefit from exploring the totality of the symptom in relation to tendon pain. Secondly, the participants in the study were individuals who did not stop training on account of their pain, they continued with their running activity regardless of the pain. Perhaps having individuals who had pain severe enough to stop them training would have helped understand the full spectrum of the pain experience.

Thirdly, while CPM is a valid tool for testing central pain mechanisms, fMRI would have been even better[696]. Additionally, for this body of work, pathology on ultrasound was not considered as exclusion. The author acknowledges that pathology without pain may alter PPTs and that this may be a potential limitation of this study. Additionally, as pain was an exclusion, only the case group completed the pain questionnaires. This is another potential limitation of the study. Another possible limitation to the study is that while the plantaris is known to be involved in midportion AT, its involvement was not screened for in this population. Lastly, the candidate gene approach to choosing genes of interest, while cost effective, is dependent on previous work and hence will not identify any novel pain genes that may well be specific to tendon pain. Hypothesis free, well powered and longitudinal genome wide association studies are needed in this field and for that genome wide association studies (GWAS), microRNA (miRNA) studies and next generation sequencing technologies such as whole genome sequencing (WGS) and whole exome sequencing (WES) may offer better options.

6.2. RECOMMENDATIONS FOR FUTURE RESEARCH AND PRACTICE

Future studies should aim to further interrogate tendon pain beyond the physical domain. This will lead to a better understanding of the lived experiences of individuals with tendon pain and hence development of more efficacious treatments. Additionally, in future work, researchers should also consider personality in the study of chronic Achilles tendon pain and the extremes of this phenotype in different cohorts. Further, other work should look at standardising imaging findings in order to improve the comparisons across studies. Standardisation of CPM protocols will also allow us to take full advantage of this technique to explore central pain mechanisms in

tendinopathy. The genetics of tendon pain is an exciting future research avenue to pursue. Using technologies such as GWAS, WGS, WES and miRNA will allow us to identify the various genes that are implicated in tendon pain. This, in turn, allows us to explore the possible biological mechanisms underlying tendon pain.

In clinical practice, multidimensional pain scales should be used to assess tendon pain as they interrogate tendon pain more extensively than the VISA-A. Additionally, tendon pain ought to be assessed, managed and treated like any other type of chronic pain. With an equal emphasis on the pain quality, its descriptors and its interferences that are beyond sporting function. Following a more holistic approach, using the biopsychosocial model of assessment and treatment may lead to improved care. The relationship between tendon structure and pain is tenuous at best, as a result, perhaps the clinical focus ought to be more towards the relationship between function and structure rather than with pain in isolation. Lastly, it is likely that central mechanisms play a key role in chronic tendinopathy, therefore, pain management protocols should reflect this with an emphasis on centrally mediated therapies instead of local, tissue-based treatments.

6.3. CONCLUSION

In conclusion, the mechanisms of pain in chronic AT are still not clearly understood. The main theories attribute the pain to inflammation, structural changes in the tendon or altered central pain mechanisms. In a novel assessment of the AT pain using the sf-BPI and the sf-MPQ, this thesis established that in this cohort, AT pain was described as aching, tender or throbbing. These word choices may be important in

establishing the underlying mechanisms in AT pain as they tend to be condition specific. Further, this thesis showed that AT pain has effects on not just sporting and physical ability but on the sufferer's mood, sleep, and relations with other people. These domains should therefore also be considered in the management of chronic AT pain. Additionally, the results of this thesis concurred with previous studies, which showed no association between tendon imaging findings and reported pain. However, through the use of the sf-BPI and the sf-MPQ, the present results showed that imaging abnormalities were associated with impaired walking ability as well as general activity. This implies that the relationship between tendon imaging, function and pain is perhaps more complex and needs to be evaluated further in order for clinicians and researchers to better understand how the three interact in pathology and in rehabilitation.

Furthermore, the results of this work are consistent with previous studies that have shown mechanical hyperalgesia in people with AT. However, no association with CPM was observed in this cohort. Another novel finding of this thesis is the association between CPM and sleep in an AT cohort, which suggests involvement of central mechanisms in chronic AT even though the CPM findings were negative. Lastly, the *COMT* rs4818 (C/G) polymorphism was associated with self-reported pain. The CC genotype of *COMT* rs4818 (C/G) was associated with higher pain scores and the GG genotype was overrepresented in the lower pain scores. This suggests an involvement of the catecholaminergic pathway in AT pain. Overall, the use of multidimensional pain scales in assessing AT yields more information on the pain report than would unidimensional ones. Therefore, future studies should evaluate tendon pain more holistically as it may help in determining its underlying mechanisms and its effects on

sufferers. Additionally, US/CD and CPM protocols are affordable means of obtaining information on tendon structure and central pain mechanisms, respectively, however, they should be standardised to allow for more reliable comparisons among studies. Lastly, studying the genetic contribution of tendon pain may help identify the mechanisms involved in AT pain as well as provide new therapeutic targets or strategies.

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APPENDIX A

Ethical approval to conduct study

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

29 September 2016

HREC REF: 279/2016

Prof M Collins
Human Biology
Sport Science Institute

Dear Prof Collins

PROJECT TITLE: AETIOLOGY OF PAIN IN CHRONIC ACHILLES TENDINOPATHY (PHD CANDIDATE - MS MKUMBUZI)

Thank you for submitting your response to the Faculty of Health Sciences Human Research Ethics Committee dated 12 September 2016.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th September 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

The HREC acknowledge that the student Sharon Nonhlanhla Mkombuzi will also be involved in this study.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

HREC 279/2016

Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

APPENDIX B

Participant recruitment flyer (cases)

WHY DO SOME ATHLETES EXPERIENCE THE PAIN OF ACHILLES TENDINOPATHY WHILE OTHERS WITH THE SAME INJURY DO NOT?

PARTICIPANTS WANTED FOR UCT RESEARCH

A comparison of the genetic differences between athletes with painful chronic Achilles tendinopathy and those who are pain free

The aim of this study is to determine the association between variations in certain genes and pain in chronic Achilles tendinopathy.

If you:

- ✓ are 18 years old or older
- ✓ with a body mass index of less than 30
- ✓ do not have a history of Achilles tendon rupture
- ✓ have not had surgery to the legs in the past two years,
- ✓ do not take any chronic medication
- ✓ Live in Cape Town

and/or

- ✓ Have pain in the Achilles tendon area for at least 3 months

You can volunteer for this study

What is involved?

- ➢ Complete a **questionnaire** and **pain rating scales**
- ➢ Donate a small blood sample (5ml=1 teaspoon)
- ➢ Have experimental cold and pressure pain induced

} once off assessment approx. 30 mins

What are the benefits?

- ➢ Receive a personalised report of and exercises for your Achilles tendinopathy

If you are interested in taking part in the study and would like additional information, please contact:

Sharon Mkumbuzi
nsmkumbuzi@gmail.com
062 340 8451

essm
EXERCISE SCIENCE AND SPORTS MEDICINE

Appendix C

Recruitment flyer (controls)

Division of Exercise Science and Sports Medicine
Department Of Human Biology, Faculty of Health Sciences
University of Cape Town, South Africa

PARTICIPANTS WANTED FOR UCT RESEARCH

A comparison of the genetic differences between athletes with painful chronic Achilles tendinopathy and those who are pain free

The **aim** of this study is to determine the association between variations in certain genes and pain in chronic Achilles tendinopathy.

If you:

- ✓ are at least 40 years old
- ✓ with a BMI of less than 30kg·m^{-2}
- ✓ do not have a history of Achilles tendon pain or tears
- ✓ have not had surgery to the legs in the past two years,
- ✓ do not take any chronic medication

You can volunteer for this study

What is involved?

- Complete a **questionnaire** and **pain rating scales**
- Donate a small blood sample (5ml=1 teaspoon)
- Have **free** **ultrasound scans** taken of your Achilles tendons
- Have experimental cold and pressure pain induced

once off assessment < 45 mins

What are the benefits?

- Receive free ultrasound scans of your Achilles tendons.

If you are interested in taking part in the study and would like additional information, please contact:

Sharon Mkumbuzi
nsmkumbuzi@gmail.com
062 340 8451

essm EXERCISE SCIENCE AND SPORTS MEDICINE

APPENDIX D

Participant information sheet and consent form

THE AETIOLOGY OF PAIN IN CHRONIC ACHILLES TENDINOPATHY

PARTICIPANT INFORMATION SHEET AND INFORMED CONSENT FORM

Dear Volunteer,

Thank you for agreeing to participate in this study to be conducted by researchers from the Division of Exercise Science and Sports Medicine within the Department of Human Biology at the University of Cape Town.

Why are we doing this study?

Achilles Tendinopathy (ATP) - pain and dysfunction in the Achilles tendon- is a common presentation in sport. The occurrence of overuse ATP in sport has risen enormously due to the increase in participation in sporting endeavours by recreational athletes and the increased duration and intensity of training regimens for professional athletes. This injury may be painless without symptoms in some individuals but often pain is the major complaint and the reason for seeking healthcare in most. However, the precise mechanisms for this pain are poorly understood. As such, most current treatment options for pain in tendinopathy are not very effective.

Some researchers have suggested that our genes may affect, at least in part, the human response to musculoskeletal pain**.** Genes are the basic units of inheritance. They are passed on from out parents and carry information on various personal characteristics such as hair colour, personality and disease risk. In an attempt to determine whether there is a genetic basis for the pain experienced in ATP and hence explain why some athletes experience it more than others, we are interested in studying whether certain genes are associated with pain in chronic tendinopathies. To do so, we will compare various features of tendons from athletes with painful tendinopathy against those with no pain.

Achilles tendinopathy, a chronic painful injury is debilitating and is challenging for clinicians and athletes to manage and we are interested in the question 'What are the differences between athletes who experience the pain of Achilles tendinopathy and those with the same injury who do not?'

What are the aims of this study?

The aims of this study are:

1. To determine the association between pain and structural changes in painful and pain free Achilles tendons using ultrasound scans.
2. To investigate the association between genetic variations in selected genes and pain in chronic Achilles tendinopathy.
3. To investigate the association between variations in selected genes and experimentally induced pain thresholds.

We anticipate that the information generated from this study will reveal which athletes are at risk of experiencing chronic Achilles tendon pain thus providing some knowledge on the possible mechanisms of pain in tendinopathy and thus allowing development of interventions to manage pain in athletes with chronic Achilles tendinopathy.

Who can take part in this study?

- If you are a physically active individual with Achilles tendon pain of at least 6 months' duration.

or

- If you are a physically active individual without Achilles tendon pain.

To volunteer you need to be above 18 years old.

If you (i) have used fluroquinolone antibiotics or corticosteroid injections to the Achilles tendon in the past 12 months (ii) have a history of Achilles tendon rupture, (iii) are taking any chronic medications (iv) have a body mass index ≥25kg·m^{-2} plus a waist circumference ≥102cm (v) have had surgery to the lower limbs in the last two years and (vi) have lower limb deformities, you shall not be eligible for this study.

What will happen if you do decide to take part in this study?

The researcher will explain the study in detail to you, including the risks and benefits associated with participating. You will be free to ask any questions you may have relating to the study. Should you agree to participate, you will sign a consent form and be asked to complete a questionnaire detailing personal information, your medical history and training. These will include questionnaires on your current pain on recruitment and the effect the pain has on your training schedule. This should take about 30 minutes to complete. On completion of these forms, a small blood sample (5ml=1 teaspoon) will be drawn from your forearm and stored in a tube until further analysis. Afterwards, the radiologist will then take ultrasound scans of your Achilles tendons, a process that should take about 15 minutes.

We also want to measure your pain thresholds to experimental pain. To do this, the investigator will ask you to immerse the hand opposite the painful tendon in ice water until the cold becomes painful. You then be asked to rate this pain on a pain scale. Following which, point pressure will then be applied to your painful Achilles tendon using a pressure probe, when this pressure becomes painful you will signal the researcher and they will stop. This should take no more than 10 minutes of your time.

What are the risks and discomforts of this study?

The potential risks associated with blood collection technique from the forearm veins are: infection, delayed healing, haematoma, physical pain, mental discomfort and injury to a nerve or a vessel. These risks are small and will be minimized by the use of trained phlebotomists, use of sterile techniques and the use of disposable, single use materials. The risks associated with the experimental pain procedure are mild discomfort from the cold water in the ice bath and from the pressure probe. However, as soon as either of these becomes uncomfortable for you, the researcher will immediately stop the protocol. The completion of questionnaires is not associated with any risk. Questionnaire and other clinical data (paper and electronic) will be kept confidential and secure, and will not be made available to any party other than the research team without your consent.

What are the benefits of taking part in this study?

There is no direct benefit to you for taking part in this study. You will however receive the results of the ultrasound scans of your Achilles tendons that you may use to inform future clinical decisions by your medical practitioner. You will also receive general feedback at the end of the study describing the genetic basis of pain in Achilles tendinopathy.

What are the ethical considerations?

The UCT Human Research Ethics Committee (contact information below) has approved this study. This study will be performed in accordance with the principles of the Declaration of Helsinki (2013, Fortaleza, Brazil), International Conference on Harmonisation and South African Good Clinical Practice (GCP) guidelines and the laws of South Africa. The study will be covered by the no-fault insurance policy of the University of Cape Town. You will not be included in the study unless you have signed a consent form, after the investigator has provided substantial verbal and written explanation of the study, including risk factors. You will be informed that your participation in the study is entirely voluntary and that you have the right to withdraw from the study at any time without stating a reason. The investigator may also withdraw you from the study at any time. All the information collected during the trial will be stored in a computer database in a secure facility, will be kept confidential and will only be used for scientific purposes. Your anonymity will be ensured should the data be published**. At the end of the study on genetic risk factors for chronic Achilles tendinopathy, any samples taken from you will be destroyed.**

What if something goes wrong?

The University of Cape Town has insurance cover for the event that research-related injury or harm results from your participation in the trial. The insurer will pay all reasonable medical expenses in accordance with the South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI) in the event of an injury or side effect resulting directly from your participation in the trial. You will not be required to prove fault on the part of the University.

The University **will not be liable** for any loss, injuries and/or harm that you may sustain where the loss is caused by:

- The use of unauthorised medicine or substances during the study
- Any injury that results from you not following the protocol requirements or the instructions that the study doctor may give you
- Any injury that arises from inadequate action or lack of action to deal adequately with a side effect or reaction to the study medication
- An injury that results from negligence on your part

By agreeing to participate in this study, you do not give up your right to claim compensation for injury where you can prove negligence, in separate litigation. In particular, your right to pursue such a claim in a South African court in terms of South African law must be ensured. Note, however, that you will usually be requested to accept that payment made by the University under the SA GCP guideline 4.11 is in full settlement of the claim relating to the medical expenses.

An injury is considered trial-related if, and to the extent that, it is caused by study activities. You must notify the study doctor immediately of any side effects and/or injuries during the trial, whether they are research-related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your injury came about because you chose not to follow the instructions that you were given while you were taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected. Copies of these guidelines are available on request.

Who do I speak to (or contact) if I have any questions about the study?

Should you have any ethical concerns or questions about the study, please contact the Human Research Ethics Committee:

Faculty of Health Sciences - Research Ethics Committee

Room E52-24, Old Main Building, Groote Schuur Hospital

Observatory, 7925

Tel: (021) 406 6338

Fax: (021) 406 6441

Email: nosi.tywabi@uct.ac.za

Should you have any queries directly related to the study itself, please contact any of the investigators:

Prof. Malcolm Collins, PhD
(021) 650 4574
malcolm.collins@uct.ac.za

A/Prof Alison September, PhD
(021) 650 4559
alison.september@uct.ac.za

N Sharon Mkumbuzi
062 340 8451
nsmkumbuzi@gmail.com

CONSENT

I, the undersigned, have been fully informed about the University of Cape Town's study entitled **"Aetiology of pain in chronic Achilles tendinopathy"** to be conducted by researchers from the Division of Exercise Science and Sports Medicine within the Department of Human Biology.

- I agree to donate five millilitres of venous blood-which will be used for the extraction and analysis of genetic material (DNA). I agree that this blood sample will be drawn by a nurse, physician or trained phlebotomist.
- I agree to complete personal particulars, sporting participation, personal and family medical history, as well as, pain rating questionnaires.
- I agree to have cold and pressure pain administered to my hand and ankle respectively
- I agree to have an ultrasound scan(s) of my Achilles tendon(s) taken.

I have been fully informed about the risks inherent in participation in this trial. I have had the opportunity to ask questions about the study and had them answered to my satisfaction. I understand that all the information collected during the study will be treated confidentially, will only be used for scientific research purposes and that my name and personal particulars will not be released under any circumstances.

I have been informed that I will be free to withdraw from the study at any time if I so wish without explanation. I will be free to ask any questions about the procedures and results of the study. I understand that I will receive, where applicable, feedback pertaining to the general results of the study once the entire study has been completed.

I agree to participate in the study.

Participant:

_____	_____	_____
Full name	Signature	Date

Investigator:

_____	_____	_____
Full name	Signature	Date

Witness:

_____	_____	_____	_____
Full name		Signature	Date

APPENDIX E

DEMOGRAPHIC QUESTIONNAIRE

Instructions

Please answer each question by filling in the details in the allocated space or checking one or more of the option boxes.

Please complete all <u>nine</u> sections A to I

Personal details form (to be completed and kept separately from the questionnaire)			
Surname			
First Name			
Postal Address		Postal/ Zip Code	
E-mail address			
Alternate E-mail address			
Cellphone number/ daytime phone			

Participant code:……

Section A: Demographic data			
Date of birth	y y y y - m m - d d		
Height	cm	Sex	Male ☐ Female ☐
Weight	kg	Age	yrs
Ethnic group (Only Required and Used for Research Purposes)	Black/African ☐ White ☐ Indian ☐ Mixed Ancestry (Coloured) ☐ Asian ☐ Other ☐		
Ancestry: Tribal or national background (eg Xhosa, Dutch, Zulu, German, Italian)	Father: Unknown ☐		
	Mother: Unknown ☐		
Country of Birth			
Dominant Hand	Left ☐ Right ☐ Both ☐	Dominant Leg	Left ☐ Right ☐ Both ☐
Current Occupation			
What **percentage** of your **working** day is spent in the following activities?	Sitting: _____ % Standing: _____ % Walking (Lower body activity) _____ % Manual Labour (upper and lower body activity) _____ %		
Occupation prior to development of tendinopathy?			
Prior to tendinopathy, did your occupation involve lower or upper limb activity?	Yes ☐ No ☐		
If yes please indicate which arms/legs.	Right arm ☐ Left arm ☐ Both arms ☐ Right leg ☐ Left leg ☐ Both legs ☐		

Section B. Sporting Details

Please record your sporting activities in order of importance

Use an additional form if you participate(d) in more than 6 sports

Type of sport(s) you have participated in (please name)	**Main sport 1**	**Other sport 2**	**Other sport 3**
Current or past participation	Current ☐ Past ☐	Current ☐ Past ☐	Current ☐ Past ☐
Year started participation			
Number of years involved in the sport			
Years in competitive sport			
Professional or amateur			
Hours of training per week (0-3 months)			
Hours of training per week (4-12 months)			
Hours of training per week (13-24 months)			

Type of sport(s) you have participated in (please name)	**Other sport 4**	**Other sport 5**	**Other sport 6**
Current or past participation	Current ☐ Past ☐	Current ☐ Past ☐	Current ☐ Past ☐
Year started participation			
Years involved in the sport			
Years in competitive sport			
Professional or amateur			
Hours of training per week (0-3 months)			
Hours of training per week (4-12 months)			
Hours of training per week (13-24 months)			

Section C. Lifestyle and habits history			
Please indicate your smoking status	Current smoker ☐	Ex smoker ☐	Never smoked ☐
If you answered yes, (past or current smoker) please complete the section on the right	Number of years of smoking:	If stopped, how many years ago:	
	What is (was) the average number of cigarettes per day:		
On average, how much alcohol do you drink per week (tots, glasses) of spirits, wine or beer?	_____ glasses beer/cider per week _____ glasses wine per week _____ tots of spirits per week		

Section D. General Personal Medical History		
Do you currently suffer from any of these medical conditions:		
☐ High Blood Pressure ☐ Emphysema ☐Malignant disease(cancer) If Yes, what type? _____	☐ Angina/Heart Attack ☐ Rheumatoid arthritis ☐ Elevated Blood Cholesterol ☐ Diabetes mellitus ☐ Renal disease	☐ Asthma ☐ Osteoarthritis (wear & tear) ☐ Adrenal disorders ☐ Thyroid disorders ☐ Amyloidosis
Do you currently suffer from any other Connective Tissue, Rheumatological Or Muscle Diseases & Disorders?	Yes ☐ No ☐	If Yes, please select from the list below
List of some Connective Tissue and/or Rheumatic Diseases and Disorders		
☐ Ankylosing Spondylitis ☐ Aspartylglycosaminuria (AGU) ☐ Behcet's Syndrome ☐ Crohn's Disease ☐ Discoid Lupus Erythematosus ☐ Ehlers-Danlos syndrome (EDS) ☐ Eosinophilic Fascitis ☐ Giant Cell (Temporal) Arthritis ☐ Gout ☐ Hypersentive Vasulatis ☐ Muscular dystrophy	☐ Lipid Storage Diseases ☐ Marfan Syndrome ☐ Menkes Kinky Hair Syndrome ☐ Mucopolysaccharidoses ☐ Myopathies and Dystrophies ☐ Ochronosis (Homocystinuria) ☐ Osteogenesis imperfecta (OI) ☐ Polyarteritis Nodosa ☐ Polymyalgia Rheumatica ☐ Polymyositis & Dermatomyositis ☐ Myopathy	☐ Pseudogout ☐ Reactive Arthritis ☐ Reiter's Syndrome ☐ Relapsing Polychrondritis ☐ Scleroderma ☐ Sjogren's Syndrome ☐ Systemic Lupus Erythematosus (SLE) ☐ Systemic Sclerosis ☐ Wegener's Granulomatosis ☐ Rhabdomyolysis ☐ Other_____

What surgical operations have you had? (please list and give dates)	Operation	Date
Do you suffer/have you suffered from (a) chronic pain condition(s)?	☐ Yes ☐ No	If yes please select from the list below

☐ Low back pain	☐ Ishaemic muscular pain	☐ Lumbago-sciatica
☐ Chronic pelvic pain	☐ Trigeminal neuralgia	☐ Vulvar pain
☐ Headaches/migraines	☐ Fibromylagia	☐ Herniated Intervertebral disc
☐ Phantom limb pain	☐ Congenital insensitivity to pain	☐ Pancreatitis
☐ Chronic widespread pain	☐ Erythromelalgia	☐ Paroxysmal extreme pain disorder
☐ Peripheral neuropathy	☐ Chronic inflammation	☐ Altered response to pain medication
☐ Increased pain sensitivity		
☐ Other (specify).....		

If female:		
At what age did you start menstruating? (years)		
Are you currently using any type of contraception?	☐ Yes ☐ No	
If Yes, what type of contraception are you using?	☐ Pill ☐ Injection ☐ IUD ☐ Rods ☐ Other (Specify)……	
Have you ever experienced labour/childbirth?	☐ Yes ☐ No	
Do you suffer/have you suffered from any painful menstrual/ hormonal conditions	☐ Yes ☐ No	If yes please select from the list below

☐ Dysmenorrhoea (period pain)
☐ Endometriosis
☐ Polycystic Ovarian Syndrome (PCOS)
☐ Other (specify)……..

Are you currently?	☐ Pre-menopausal (±12 cycles per year at intervals of 23– 33 days & bleeding lasts 3-7 days) ☐ Menopausal (cycles are irregular and less frequent) ☐ Post-menopausal (no longer menstruating)

Section E. Family Medical History

Have any of your blood (biological) relatives <u>ever</u> had the following?

Please tick yes or no. If yes, please tick the relationship of that person to you (You may tick more than one of the relationship blocks).

Description		**If Yes, please indicate the relationship**

Chronic **Achilles** tendon injury	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Achilles tendon rupture	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Any other (not Achilles) tendon injury/rupture	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Any ligament injury	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Exercise associated muscle cramps	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Night muscle cramps	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Do any other members of your family suffer from elevated blood cholesterol?	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Is there any history of arthritis in your family?	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother

Heart Disease	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother
Diabetes	Yes ☐ No ☐	☐ Father ☐ Mother ☐ Brother ☐ Sister ☐ Child ☐ Grandfather ☐ Grandmother

Section F. History of Medication Use			
What medication, if any (including painkillers), are you currently using? (please list)	Name of medication		Days/Months/Years taken
Have you ever used oral corticosteroids (cortisone tablets)? (If **yes**, how long ago?)	Yes ☐ No ☐	☐ 3 months ☐ 12 months	☐ 6 months ☐ 24 or more months
Have you ever been given an injection with corticosteroids? (If **yes**, how long ago?)	Yes ☐ No ☐	☐ 3 months ☐ 12 months	☐ 6 months ☐ 24 or more months
Have you ever used fluoroquinoline antibiotics? (refer to the following list)	Yes ☐ No ☐	☐ 3 months ☐ 12 months	☐ 6 months ☐ 24 or more months

List of some fluoroquinoline antibiotics (may be used in treatment of chlamydia, pneumonia, acute bronchitis, urinary tract infections, skin and soft tissue infection):		
ADCO-CIPRIN	CIPROBAY	SANDOZ CIPROFLOXACIN
AVELON	CIPROGEN	TAFLOC
BACTIDRON	CPL ALLIANCE CIPROFLOXACIN	TARIVID
CIFLOC	DYNAFLOC	TAVANIC
CIFRAN	FACTIVE	TEQUIN
CIPLA-CIPROFLOXACIN	FLOXIN	UNIQUIN
CIPLOXX	MAXAQUIN	UTIN-400
CIPRO-HEXAL	NOROXIN	ZANOCIN
	ORPIC	

Section G. Past History of Tendon, Ligament or Joint Capsule Injury

Please complete this section for each injury. If you have had more than one past injury additional forms will be available.

Have you **ever** in your suffered from **a tendon or ligament injury** (pain, swelling, stiffness) in any tendon (including Achilles tendon, knee tendons, and shoulder tendons) or ligaments (partial or complete tear)?	Yes ☐ No ☐

If **YES,** please complete the rest of the section below:-

If **NO**, continue completing the questionnaire from section L.

		Tendon	Longstanding Pain **(Tendinopathy)** **Left Right**	Acute Tear/ **Rupture** **Left Right**
Please tick which **tendon/s** you have injured? (next column on the tright) Also indicate (tick) if your injured tendon was longsatnding pain (tendinopathy) or an acute tear/rupture	Foot and ankle:	☐ Achilles tendon	☐☐	☐☐
		☐ Tibialis posterior	☐☐	☐☐
		☐ Plantar fascia	☐☐	☐☐
	Knee:	☐ Patellar tendon	☐☐	☐☐
	Elbow and wrist:	☐ Wrist extensor tendon	☐☐	☐☐
	Shoulder:	☐ Rotator cuff	☐☐	☐☐
	Other: _____		☐☐	☐☐

	Ligament	Sprain **Left Right**	**Complete Tear Left Right**
Please tick which **ligament/s** you have injured? (next column on the tright) Also indicate if your sprained or completely tore the ligamant.		☐☐	☐☐
	☐ Shoulder ligaments	☐☐	☐☐
	☐ Elbow ligaments	☐☐	☐☐
	☐ Wrist ligaments	☐☐	☐☐
	☐ Finger ligaments	☐☐	☐☐
	☐ Knee (ACL)	☐☐	☐☐
	☐ Knee (MCL)	☐☐	☐☐
	☐ Knee (PCL)	☐☐	☐☐
	☐ Knee (LCL)	☐☐	☐☐
	☐ Ankle lateral ligaments	☐☐	☐☐
	☐ Ankle medial ligaments	☐☐	☐☐
	☐ Spinal ligaments	☐☐	☐☐
	☐ Other: _____	☐☐	☐☐

Have you ever suffered from any of the following joint capsule injuries?	☐ Acute shoulder dislocation ☐ Chronic shoulder instability ☐ Chronic ankle instability ☐ Other: _____

SECTION H. MEDICAL DETAILS OF TENDON INJURIES				
Symptoms				
How many times have you had tendon injuries? [1]Sudden onset is within a few seconds or minutes [2]Gradual onset is over days or weeks	Tendon Injured	Date of Injury	Acute or Chronic Injury	Sudden[1] or Gradual[2] Onset
	1			
	2			
	3			
	4			
	5			

Please complete this form for each tendon Injury you have had			
Injury Number (1,2,3,4,or 5)	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ _____		
Which tendon did you injure?	Rotator cuff tendon ☐ • Supraspinatus ☐ • Infraspinatus ☐ • Teres minor ☐	Patellar tendon ☐ Wrist extensor tendons ☐ Achilles tendon ☐	
Which side was injured?	☐ Left	☐ Right	☐ Both
Which region of your tendon was injured? Please indicate on a diagram. (Only if applicable)	☐ Upper 1/3	☐ Middle 1/3	☐ Lower 1/3
To what extent was your Tendon ruptured?	☐ Complete	☐ Partial	☐ None
How were you injured? (e.g. sport, walking)			

Grade of injury **at the time** of injury	□ pain only after exercise □ pain during exercise, but did not cause you to alter training □ pain during exercise, which causes you to alter training □ pain which causes you to stop training □ no pain □ not sure □ Other (Specify _____)
Grade of injury **currently**	□ pain only after exercise □ pain during exercise, but did not cause you to alter training. □ pain during exercise, which causes you to alter training □ pain which causes you to stop training □ no pain □ not sure □ Other (Specify) _____
Which of the following symptoms were present **before** the injury	□ Pain (less than 1 week) □ Stiffness □ Pain (1-4 weeks) □ Swelling □ Pain (> 4 weeks) □ None
Which of the following symptoms were present **after** the injury	□ Pain (less than 1 week) □ Stiffness □ Pain (1-4 weeks) □ Swelling □ Pain (> 4 weeks) □ None
Diagnosis and treatment	
Which type of Tendon Disease were you diagnosed with e.g. Rupture, Tendinitis, etc.	
Diagnosed by	□ Doctor _____ □ Physiotherapist _____ □ Biokineticist _____ □ Podiatrist _____ □ Other _____

How was it treated?	□ Surgically □Non-surgically	If non-surgically please select from the list which modality (s) alleviated the pain
□ oral NSAIDs (such as ibuprofen, diclofenac, indomethacin) □ oral corticosteroids □ injected NSAIDs (such as diclofenac) □ plasma rich platelet injections □ sclerosants (such as polidoconal □ eccentric exercise □ electrotherapies (such as ultrasound, TENS, ShortWave Diathermy) □ heat therapy □ ice □ Extracoporeal shockwave therapy (ESWT) □ orthotics □ Acupunture □ rest □ taping/strapping □ massage □ activity modification □ Other (specify)……		

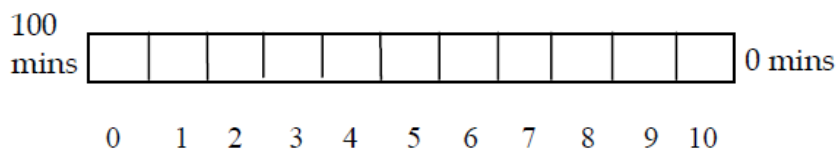
APPENDIX F

VISA-A Questionnaire

The VISA-A questionnaire: An index of the severity of Achilles tendinopathy

IN THIS QUESTIONNAIRE, THE TERM PAIN REFERS SPECIFICALLY TO PAIN IN THE ACHILLES TENDON REGION

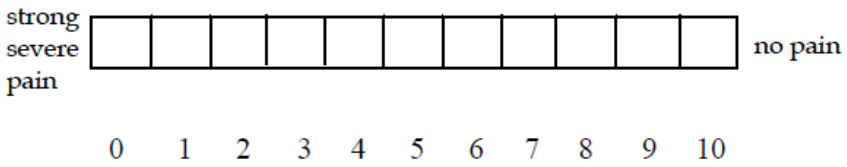
1. For how many minutes do you have stiffness in the Achilles region on first getting up?



POINTS

□

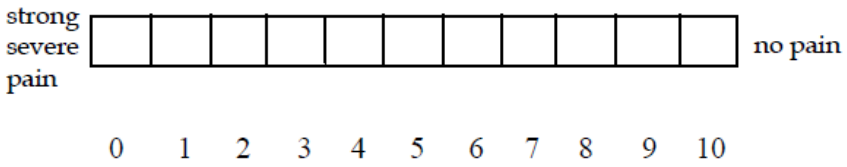
2. Once you are warmed up for the day, do you have pain when stretching the Achilles tendon fully over the edge of a step? (keeping knee straight)



POINTS

□

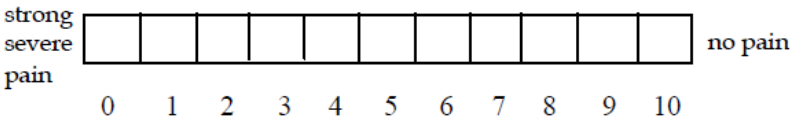
3. After walking on flat ground for 30 minutes, do you have pain within the next 2 hours? (If unable to walk on flat ground for 30 minutes because of pain, score 0 for this question).



POINTS

□

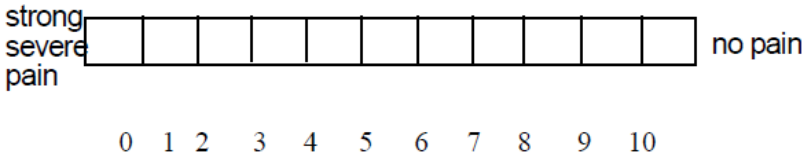
4. Do you have pain walking downstairs with a normal gait cycle?



POINTS

□

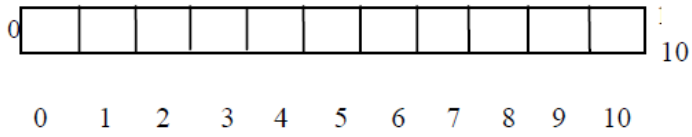
5. Do you have pain during or immediately after doing 10 (single leg) heel raises from a flat surface?



POINTS

□

6. How many single leg hops can you do without pain?



POINTS

□

7. Are you currently undertaking sport or other physical activity?

0	□	Not at all	POINTS
4	□	Modified training ± modified competition	□
7	□	Full training ± competition but not at same level as when symptoms began	
10	□	Competing at the same or higher level as when symptoms began	

8. Please complete **EITHER A, B or C** in this question.

- If you have **no pain while undertaking Achilles tendon loading sports** please complete **Q8a only**.
- If you have **pain while undertaking Achilles tendon loading sports but it does not stop you from completing the activity**, please complete **Q8b only**.
- If you have **pain that stops you from completing Achilles tendon loading sports**, please complete **Q8c only**.

A. If you have **no pain** while undertaking **Achilles tendon loading sports**, for how long can you train/practise?

NIL	1-10 mins	11-20 mins	21-30mins	>30 mins	POINTS
□	□	□	□	□	□
0	7	14	21	30	

OR

B. If you have some pain while undertaking **Achilles tendon loading sport**, but it does not stop you from completing your training/practice for how long can you train/practise?

NIL	1-10 mins	11-20 mins	21-30mins	>30 mins	POINTS
□	□	□	□	□	□
0	4	10	14	20	

OR

C. If you have **pain that stops you** from completing your training/practice in **Achilles tendon loading sport**, for how long can you train/practise?

NIL	1-10 mins	11-20 mins	21-30mins	>30 mins	POINTS
□	□	□	□	□	□
0	2	5	7	10	

TOTAL SCORE (/100) □ %

APPENDIX G

Short form McGill Pain Questionnaire

SHORT FORM McGILL PAIN QUESTIONNAIRE

Please rate your pain by marking the box besides the word that best describes your pain and its severity at present.

	None	Mild	Moderate	Severe
Throbbing				
Shooting				
Stabbing				
Sharp				
Cramping				
Gnawing				
Hot-burning				
Aching				
Heavy				
Tender				
Splitting				
Tiring-Exhausting				
Sickening				
Fearful				
Punishing-Cruel				

Present pain intensity (please circle)

0 no pain
1 mild
2 discomforting
3 distressing
4 horrible
5 excruciating

APPENDIX H

Short form Brief Pain Inventory

1903

Date: ☐☐ / ☐☐ / ☐☐
(month) (day) (year)

Subject's Initials : _____

Study Subject #: ☐☐☐☐

PLEASE USE BLACK INK PEN

Study Name: _____

Protocol #: _____

PI: _____

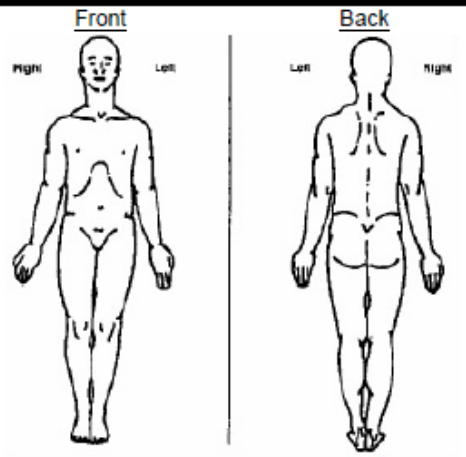
Revision: 07/01/05

Brief Pain Inventory (Short Form)

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these everyday kinds of pain today?

☐ Yes ☐ No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain by marking the box beside the number that best describes your pain at its worst in the last 24 hours.

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

No Pain — Pain As Bad As You Can Imagine

4. Please rate your pain by marking the box beside the number that best describes your pain at its least in the last 24 hours.

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

No Pain — Pain As Bad As You Can Imagine

5. Please rate your pain by marking the box beside the number that best describes your pain on the average.

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

No Pain — Pain As Bad As You Can Imagine

6. Please rate your pain by marking the box beside the number that tells how much pain you have right now.

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

No Pain — Pain As Bad As You Can Imagine

Page 1 of 2

1903

Date: ___ / ___ / ___ (month) (day) (year)

Subject's Initials : _____

Study Subject #: ____

PLEASE USE BLACK INK PEN

Study Name: _____

Protocol #: _____

PI: _____

Revision: 07/01/05

7. What treatments or medications are you receiving for your pain?

8. In the last 24 hours, how much relief have pain treatments or medications provided? Please mark the box below the percentage that most shows how much relief you have received.

0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
☐ No Relief	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐ Complete Relief

9. Mark the box beside the number that describes how, during the past 24 hours, pain has interfered with your:

A. General Activity

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

B. Mood

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

C. Walking ability

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

D. Normal Work (includes both work outside the home and housework)

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

E. Relations with other people

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

F. Sleep

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

G. Enjoyment of life

☐ 0 Does Not Interfere ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Completely Interferes

APPENDIX I

Permission from co- authors to use manuscripts in thesis

I, the undersigned **Dr BRENDA OULO** as co-author to the manuscript(s) listed below, grant **Nonhlanhla Sharon Mkumbuzi** the permission to include the following manuscripts in her Doctoral thesis.

1. Mkumbuzi, N.S., September, A.V., Posthumus, M., Oulo, B., Mafu, T.S., & Collins, M. Characterisation of Achilles tendon pain using multidimensional pain scales. *Journal of Science and Medicine in Sport.* **2020**

Signed... ***..... Date.....10-07-2020.....***

I, the undersigned **DR OSCAR HOCH JØRGENSEN** as co-author to the manuscript(s) listed below, grant **Nonhlanhla Sharon Mkumbuzi** the permission to include the following manuscripts in her Doctoral thesis.

1. Mkumbuzi, N.S., Jørgensen, O., Mafu, T.S., September, A.V., Posthumus, M.,& Collins, M. Tendon structure is not related to self-reported symptoms in recreational runners with chronic Achilles tendinopathy. *Translational Sports Medicine*. ***In press***

***Signed**.. . .. … ……. Date: 13-07-2020*

To whom it may concern

I, the undersigned **SENANILE B DLAMINI** as co-author to the manuscript(s) listed below grant **Nonhlanhla Sharon Mkumbuzi** the permission to include the following manuscripts in her Doctoral thesis.

1. Mkumbuzi, N.S., Dlamini, S.B., Mafu, T.S., Posthumus, M., September, A.V., & Collins, M. A novel evaluation of *COMT, SCN9A, TAC1* and *TACR-1* polymorphisms in a South African cohort of recreational athletes with chronic Achilles tendinopathy highlights an association between *COMT*, pain sensitivity and self-reported tendon pain. ***Under review***

I further confirm that I will not be including this manuscript(s) in my Doctoral thesis.

Signed..... ..... ***Date....***10/8/2020

To whom it may concern

I, the undersigned **TREVOR S MAFU** as co-author to the manuscript(s) listed below, grant **Nonhlanhla Sharon Mkumbuzi** the permission to include the following manuscripts in her Doctoral thesis.

1. Mkumbuzi, N.S., September, A.V., Posthumus, M., Oulo, B., Mafu, T.S., & Collins, M. Characterisation of Achilles tendon pain using multidimensional pain scales. *Journal of Science and Medicine in Sport.* **2020**
2. Mkumbuzi, N.S., Jorgensen, O., Mafu, T.S., September, A.V., Posthumus, M.,& Collins, M. Tendon structure is not related to self-reported symptoms in recreational runners with chronic Achilles tendinopathy. *Translational Sports Medicine*. ***2020***
3. Mkumbuzi, N.S., Mafu, T.S., September, A.V., Posthumus, M.,& Collins, M. Conditioned pain modulation is not altered in recreational athletes with chronic Achilles tendinopathy. ***Under review***
4. Mkumbuzi, N.S., Dlamini, S.B., Mafu, T.S., Posthumus, M., September, A.V., & Collins, M. A novel evaluation of *COMT, SCN9A, TAC1* and *TACR-1* polymorphisms in a South African cohort of recreational athletes with chronic Achilles tendinopathy highlights an association between *COMT*, pain sensitivity and self-reported tendon pain. ***Under review***

I further confirm that I will not be including these manuscripts in my Doctoral thesis.

Signed……..…… ……. Date…10 August 2020.

To whom it may concern

I, the undersigned ……**Michael Posthumus**……., as co-author to the manuscript(s) listed below, grant **Nonhlanhla Sharon Mkumbuzi** the permission to include the following manuscripts in her Doctoral thesis.

1. Mkumbuzi, N.S., September, A.V., Posthumus, M., Oulo, B., Mafu, T.S., & Collins, M. Characterisation of Achilles tendon pain using multidimensional pain scales. *Journal of Science and Medicine in Sport.* **2020**
2. Mkumbuzi, N.S., Jorgensen, O., Mafu, T.S., September, A.V., Posthumus, M.,& Collins, M. Tendon structure is not related to self-reported symptoms in recreational runners with chronic Achilles tendinopathy. *Translational Sports Medicine.* ***2020***
3. Mkumbuzi, N.S., Mafu, T.S., September, A.V., Posthumus, M.,& Collins, M. Conditioned pain modulation is not altered in recreational runners with chronic Achilles tendinopathy: A case-control study. ***Under review***
4. Mkumbuzi, N.S., Dlamini, S.B., Mafu, T.S., Posthumus, M., September, A.V., & Collins, M. A novel evaluation of *COMT, SCN9A, TAC1* and *TACR-1* polymorphisms in a South African cohort of recreational athletes with chronic Achilles tendinopathy highlights an association between *COMT*, pain sensitivity and self-reported tendon pain. ***Under review***

Signed…… Date…12/08/2020

To whom it may concern

I, the undersigned ……Alison V Septembe……, as co-author to the manuscript(s) listed below, grant **Nonhlanhla Sharon Mkumbuzi** the permission to include the following manuscripts in her Doctoral thesis.

1. Mkumbuzi, N.S., September, A.V., Posthumus, M., Oulo, B., Mafu, T.S., & Collins, M. Characterisation of Achilles tendon pain using multidimensional pain scales. *Journal of Science and Medicine in Sport*. **2020**
2. Mkumbuzi, N.S., Jorgensen, O., Mafu, T.S., September, A.V., Posthumus, M.,& Collins, M. Tendon structure is not related to self-reported symptoms in recreational runners with chronic Achilles tendinopathy. *Translational Sports Medicine*. ***2020***
3. Mkumbuzi, N.S., Mafu, T.S., September, A.V., Posthumus, M.,& Collins, M. Conditioned pain modulation is not altered in recreational runners with chronic Achilles tendinopathy: A case-control study. ***Under review***
4. Mkumbuzi, N.S., Dlamini, S.B., Mafu, T.S., Posthumus, M., September, A.V., & Collins, M. A novel evaluation of *COMT, SCN9A, TAC1* and *TACR-1* polymorphisms in a South African cohort of recreational athletes with chronic Achilles tendinopathy highlights an association between *COMT*, pain sensitivity and self-reported tendon pain. ***Under review***

Signed ***Date……11 August 2020……***

To whom it may concern

I, the undersigned, **MALCOLM COLLINS**, as co-author to the manuscript(s) listed below, grant **Nonhlanhla Sharon Mkumbuzi** the permission to include the following manuscripts in her Doctoral thesis.

1. Mkumbuzi, N.S., September, A.V., Posthumus, M., Oulo, B., Mafu, T.S., & Collins, M. Characterisation of Achilles tendon pain using multidimensional pain scales. *Journal of Science and Medicine in Sport.* **2020**
2. Mkumbuzi, N.S., Jorgensen, O., Mafu, T.S., September, A.V., Posthumus, M.,& Collins, M. Tendon structure is not related to self-reported symptoms in recreational runners with chronic Achilles tendinopathy. *Translational Sports Medicine.* ***2020***
3. Mkumbuzi, N.S., Mafu, T.S., September, A.V., Posthumus, M.,& Collins, M. Conditioned pain modulation is not altered in recreational runners with chronic Achilles tendinopathy: A case-control study. ***Under review***
4. Mkumbuzi, N.S., Dlamini, S.B., Mafu, T.S., Posthumus, M., September, A.V., & Collins, M. A novel evaluation of *COMT, SCN9A, TAC1* and *TACR-1* polymorphisms in a South African cohort of recreational athletes with chronic Achilles tendinopathy highlights an association between *COMT*, pain sensitivity and self-reported tendon pain. ***Under review***

Signed…… ***……. Date……*** *9 August 2020* ***…….***