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Pain Duration is Associated with Increased Muscle Sympathetic Nerve Activity in Patients with Achilles Tendinopathy

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Abstract

Introduction: Tendinopathy is a common condition, which has been linked to surrogate measures of sympathetic nervous system (SNS) activity and insulin resistance. This study aimed to compare in vivo measures of the SNS and insulin resistance between individuals with and without Achilles tendinopathy.

Methods: This case-control study compared Achilles tendinopathy sufferers to healthy controls. SNS activity was quantified using muscle sympathetic nerve activity (MSNA), while metabolic status was assessed via a modified glucose tolerance test and fasting lipid panel. Ultrasound tissue characterisation assessed tendon structure.

Results: Resting MSNA did not differ between the 15 cases and 20 controls. Tendon pain duration in tendinopathy patients was correlated with burst frequency ($R^2 = 0.32$, $p = 0.02$) and burst incidence ($R^2 = 0.41$, $p = 0.01$) of MSNA. After adjusting for multiple comparisons, there was a trend suggesting fasting glucose was greater in cases (median 4.80, IQR 0.70 in cases vs 4.51, 0.38 in controls) and correlated with pain severity ($R^2 = 0.14$, $p = 0.03$), but no other metabolic measures were associated with tendon pain/structure.

Conclusions: This study indicates that SNS activity is associated with tendon pain duration, building on previous data indicating the SNS is involved in recalcitrant tendinopathy. Metabolic parameters had little relationship with Achilles tendinopathy in this metabolically homogenous sample. Prospective studies are required to uncover the precise relationship between SNS activity, insulin resistance and tendinopathy.

Key Words

MSNA, tendons, metabolic, UTC.

1. Introduction

Tendinopathy is a clinical diagnosis of tendon pain and reduced function (Maffulli et al. 2003). The underlying pathophysiology is not completely understood and so clinical management remains a challenge (Maffulli, Wong 2003; Rio et al. 2014). The current understanding is that the pain experience is not solely explained by centrally mediated pain or peripheral nociceptive pain (Rio, Moseley 2014). For this reason, new factors are being studied in tendon pain. In the past decade, both the sympathetic nervous system (SNS) and insulin resistance have been increasingly implicated as potential contributors to the process of pain, reduced function and possibly poorer tendon structure (Gaida et al. 2009; Jewson et al. 2015).

Overactivity of the sympathetic nervous system (SNS) has been identified as a factor associated with tendinopathy (Jewson, Lambert 2015). Markers of sympathetic activity are upregulated in the paratendinous tissue and on abnormal tenocytes of painful tendons (Bjur et al. 2008; Danielson et al. 2007; Tosounidis et al. 2013; Zeisig et al. 2009). These markers appear to depend on the tissue type being studied. Several studies demonstrated that markers of sympathetic innervation were not changed in tendinopathic biopsies from the tendon proper (as opposed to paratendinous tissue) (Bjur, Danielson 2008; Danielson, Alfredson 2007; Ljung et al. 1999; Zeisig, Ljung 2009), and one study identified that SNS markers were actually downregulated in this tissue (Lian et al. 2006). To date, markers of sympathetic activity have only been measured via immunohistochemistry (Jewson, Lambert 2015), which does not measure actual *in vivo* sympathetic nerve activity.

Type 2 diabetes mellitus (T2DM) is a risk factor for tendon rupture (Zakaria et al. 2014) and is similarly associated with tendinopathy (Ranger et al. 2015). Individuals with diabetes have

thicker tendons and are more likely to have tendinopathy, while people with diabetes and tendinopathy have longer duration of diabetes than those with diabetes but without tendinopathy (Ranger, Wong 2015). Furthermore, a study of individuals with Achilles tendinopathy showed that, in comparison to well-matched controls, they had lipid profiles indicative of insulin resistance, which is the key metabolic defect underlying T2DM (Gaida, Alfredson 2009). However, like studies on the SNS and tendinopathy, no true *in vivo* markers of insulin resistance have ever been studied in tendinopathy. Therefore, debate continues as to whether these changes are due to the metabolic state of insulin resistance or simply elevated glucose levels.

Finally, upregulation of the SNS is known to contribute to acute and chronic pain via peripheral and central methods (Burton et al. 2016; Schlereth & Birklein 2008; Torebjork et al. 1995) and precede the development of insulin resistance (Flaa et al. 2008; Masuo et al. 1997). These interrelationships and strong circumstantial evidence implicating both the SNS and insulin resistance in the pathophysiology of tendinopathy point to a significant knowledge gap; no study has recorded *in vivo* SNS activity or a direct measure of insulin resistance in tendinopathy. Therefore, the aim of this study was to compare *in vivo* SNS activity and insulin resistance among individuals with and without Achilles tendinopathy.

2. Methods

2.1 Study design and recruitment

Both the Monash University Human Research Ethics Committee (approval number CF13/3543 – 2013001776) and the Alfred Health Human Ethics Committee (approval number 114/14) granted ethics approval for this case-control study. Participants were

recruited from the Melbourne metropolitan area according to the eligibility criteria (table 1) and provided written informed consent. Financial reimbursement up to AU\$100 was provided for travel expenses.

<Please insert table 1 here>

2.2 Data Collection

Participants provided a basic medical history and history of medication, alcohol, tobacco and recreational drug use. Height and weight were measured to calculate body mass index (BMI) and the waist-to-hip circumference ratio was measured. Resting blood pressure (BP) was recorded (Digital Automatic Blood Pressure Monitor HEM907, Omron Electronics Pty Ltd) using a mean of three readings across five minutes in each participant.

Two participant questionnaires were administered. The first assessed physical activity (including running kilometres per week and running years) and basic tendon pain characteristics (e.g. pain duration, presence of physical trauma to tendon, diagnosis). The second was the Victorian Institute of Sport Assessment – Achilles (VISA-A), which is used to provide a reliable, quantifiable measure of Achilles tendon pain (Robinson et al. 2001). These were performed on the first of the testing days. SNS recordings were performed within 1-2 weeks of tendon structure analysis, which were followed by metabolic parameter testing 1-2 weeks later.

Sympathetic nervous system measurements

Subjects fasted for 4-5 hours and avoided caffeine for 18 hours prior to the recording. Participants were instructed not to take any regular medication on the morning of the recording (excluding the oral contraceptive pill).

ACHILLES PAIN DURATION ASSOCIATED WITH MSNA

Supine recordings of multi-unit postganglionic muscle sympathetic nerve activity (MSNA) were made using a tungsten microelectrode (FHC, Bowdoinham, ME, USA) inserted directly into the peroneal nerve at the fibular head according to previously described methods (Lambert et al. 2007). This technique is the gold-standard method to measure sympathetic activity of the muscle vasoconstrictor outflow (Grassi & Esler 1999). The right peroneal nerve was recorded in the control group. In the case group, the peroneal nerve of the contralateral limb to the painful Achilles tendon was measured. If both Achilles tendons were painful, the nerve of the least painful limb was recorded and if the tendons were equally painful, the right peroneal nerve was recorded. This was to ensure that the most painful tendon could have a painful stimulus without interfering with the recording (see below). A subcutaneous reference electrode was positioned 2 to 3 cm away from the recording site. The nerve signal was amplified (x50,000), filtered (bandpass, 700 to 2000Hz), and integrated.

Beat to beat BP was measured in the finger using a Finometer device (model Datex-Ohmeda 2300, Louisville, USA). Heart rate was derived from continuous 3-lead ECG recordings. BP, ECG, and MSNA signals were digitized at 1000Hz (PowerLab, model ML 785/8SP, ADI Instruments, NSW, Australia). Resting measurements were recorded over a 10-minute period and averaged.

After resting recordings were taken, the Achilles tendon contralateral to MSNA recording was squeezed for ten seconds with a Harpenden skinfold calliper at a standardised force of 10g/mm² and contact area of 0.75cm². This was performed at the site of pain in the case group, or in the mid-portion of the tendon (2-6cm from the calcaneus) in the control group, in order to assess the SNS response to pain/pressure, which has been previously described in studies on mechanical and noxious stimuli (Fazalbhoy et al. 2012; Schobel et al. 1996).

ACHILLES PAIN DURATION ASSOCIATED WITH MSNA

MSNA recordings were taken for the 10 minutes following the tendon squeeze and participants reported pain on a 100mm visual analogue scale.

Metabolic parameters

Participants also underwent a modified oral glucose tolerance test (OGTT) and a lipid panel at a blood collection centre following an eight-hour overnight fast. The 3-sample OGTT involved participants drinking a 75g glucose drink before having blood collected (via standard, aseptic venepuncture) at 0, 60 and 120 minutes. The OGTT was modified with the addition of insulin measurement at each time point. The fasting lipid panel included total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C).

Ultrasound tissue characterisation (UTC)

Achilles tendon structure was semi-quantifiably measured in both tendons using a UTC scanner (UTC imaging, Kruisstraat, Netherlands). This method has been validated against tendon biopsies in horses (van Schie & Bakker 2000), shows structural changes in painful human tendons (van Schie et al. 2010), and is responsive to maximal exercise (Docking & Cook 2015). A 10-MHz linear-array ultrasound transducer (Smartprobe 10L5; Terason 2000, Teratech, USA), was automatically moved by the tracker (UTC imaging, Kruisstraat, Netherlands) along the length of the tendon. 600 transverse ultrasound images were taken over a length of 12cm (i.e. a transverse image was taken every 0.2mm) and assembled via software supplied with the UTC device to produce a three dimensional image (UTC 2010; UTC Imaging).

All participants were instructed to stand on a box that was 57cm tall, with the first toe and knee of the scanned leg touching the wall in front of them. This ensured a standardised position of the scanned leg for each participant. The UTC scanner was placed on the tendon, aligned with long-axis of the tendon, and the tracking device triggered, which moved the ultrasound probe proximally to capture images.

2.3 Data analysis

Sympathetic nervous system measurements

Participant ID numbers associated with data files were replaced with computer-generated random numbers so that the researcher performing data analysis was blind to group allocation and experimental condition. MSNA was analysed at rest, and the analysis of the 10-minute recording post the tendon squeeze was separated into the first and latter five minutes periods.

MSNA was analysed manually, by visual inspection of the neurogram using the LabChart computer program (Version 5.5.5, ADI Instruments). MSNA was expressed as burst frequency (bursts/min) and burst incidence (bursts/100 heartbeats). Burst amplitude was measured by normalising MSNA using the largest burst detected at rest being designated as 100. Burst amplitude was then expressed as a percentage of this value. Total MSNA was thus calculated as units/min (bursts/min x mean burst amplitude) and units/100 heartbeats (bursts/100 heartbeats x mean burst amplitude), giving 4 measures of MSNA (burst frequency, burst incidence, units/min and units/100 heartbeats). This analysis was carried out by a sole investigator (JJ) with confirmation provided by EL. Variability associated with one-investigator performing repeated analysis of the same trace is 6%.

Metabolic parameters

Fasting glucose and insulin from the OGTT were used to calculate the Matsuda index for insulin sensitivity, an index highly correlated with the euglycaemic-hyperinsulinaemic clamp (Matsuda & DeFronzo 1999). The peak glucose reading was also noted. In addition to the lipid panel, ratios were calculated for TC-to-HDL-C, LDL-C-to-HDL-C and TG-to-HDL-C; the TG-to-HDL-C ratio is particularly important when studying insulin resistance, and is strongly correlated with LDL-C particle diameter (Gaida, Alfredson 2009; McLaughlin et al. 2005).

Ultrasound tissue characterisation

File names were replaced with random, computer-generated numbers so analysis could be conducted blind to group allocation. The most painful tendon was analysed in each of the participants with tendinopathy, while the right tendon was analysed in the control participants. The region of analysis extended from the disappearance of the calcaneus to the appearance of the triceps surae muscles. Each ultrasound scan was examined to identify any obvious pathology. Each image was then contoured by outlining the tendon on the transverse plane at regular intervals along the length of the tendon (no more than 0.5mm apart). A tendon volume was created automatically by interpolating between the selected contours. Finally, the UTC algorithm was run using dedicated software (UTC2010; UTC Imaging), which gave an output of the percentage composition for each of the 4 different echo-types within the tendon. These echo-types represent tendon structure, where echotypes 1 and 2 denote aligned fibrillar structure and echotypes 3 and 4 represent disorganised tendon structure (Docking et al. 2015; Rosengarten et al. 2015; van Schie, de Vos 2010).

Statistics

Group differences in parametric SNS data were tested using independent t-tests, while Mann-Whitney U tests were used for non-normally distributed data. Analysis of post squeeze data was compared using 2-way ANOVA, in order to test whether there were differences in MSNA response following the tendon squeeze. Differences in categorical data were tested using chi-square tests. Bivariate correlation analyses were performed between continuous parameters using the non-parametric, two-tailed Spearman rank correlations and controlled for group where necessary. Statistical significance was accepted at a p value of <0.05 , with Bonferonni adjustments for multiple comparisons where required (Graphpad Prism version 6, GraphPad Software Inc; IBM SPSS Statistics version 22, IBM Corporation).

3. Results

3.1 Participant details

<Please insert table 2 here>

The 15 case and 20 control participants were young adults with healthy BMI (table 2). The median pain duration of cases was 24 months while most tendinopathies were mid-portion (table 3). Of the 5 cases who had not experienced pain within the last week, the average length of time since the last bout of pain was 4.8 weeks (range of 2-8 weeks).

<Please insert table 3 here>

3.2 Sympathetic nervous system

Microneurography recordings were successfully obtained at baseline for all 15 cases and 17 of the 20 controls; this rate of technical failure is within normal limits for this procedure.

<Please insert table 4 here>

Resting values of burst frequency ($R^2 = 0.324$, $p = 0.027$) and burst incidence ($R^2 = 0.408$, $p = 0.010$) were both positively correlated with tendon pain duration. Units per minute ($R^2 = 0.207$, $p = 0.089$) and units per 100 heartbeats ($R^2 = 0.228$, $p = 0.071$) at rest showed a trend towards a correlation with pain duration. No correlation was found between VISA-A and any microneurography measure.

More cases experienced tendon pain post squeeze compared with controls (12 vs. 3, $p = 0.0004$). All 15 MSNA traces from cases were suitable for post-squeeze analysis, while 13 of the 17 controls had suitable post-squeeze traces. There was no significant change in any of the MSNA parameters between baseline and the first five minutes of analysis post squeeze or between baseline and the latter five minutes of analysis post squeeze (data not shown). There was also no significant difference in the MSNA response to the squeeze when comparing groups.

3.3 Metabolic factors

After allowing for multiple comparisons there was a trend for fasting glucose to be significantly higher in cases than controls, however fasting insulin, the Matsuda index and lipid profile values/ratios were not significantly different (table 5). Fasting glucose values were negatively, although not strongly, correlated with VISA-A scores, ($R^2 = 0.140$, $p = 0.027$). Maximum glucose during the OGTT, fasting insulin, Matsuda index and lipid profile were not correlated with VISA-A scores and none of the metabolic measures listed were correlated with tendon pain duration ($R^2 = 0.0002 - 0.228$, $p = 0.071 - 0.962$).

<Please insert table 5 here>

3.4 UTC analysis

There were significantly more pathological tendons (i.e. the presence of a hypoechogenicity) in the case group (6/15) than the control group (0/20) ($p = 0.002$). Tendon antero-posterior (AP) diameter was greater in the case group than the control group (cases = $6.7 \pm 1.9\text{mm}$, controls = $5.9 \pm 1.1\text{mm}$, median $\pm$ IQR; $p = 0.04$). Quantifiable UTC analysis revealed no difference in percentage of echo-types 1 and 2 ($p = 0.40$) and echo-types 3 and 4 ($p = 0.52$) between groups.

No resting MSNA measures were correlated with UTC echotypes 1 and 2. It was noted, however, that there was a significant outlier in this data (figure 1), which was present with all MSNA measures. Data were therefore also analysed without this outlier.

<Please insert figure 1 here>

Analysis of the data excluding this outlier had an impact on the findings. When controlling for group (cases or controls) in the correlation analysis, all MSNA measures were negatively correlated with echo-types 1 and 2, i.e. as MSNA increased, aligned fibrillar structure decreased (burst frequency $R^2 = 0.144$, $p = 0.039$; burst incidence $R^2 = 0.167$, $p = 0.025$; units per minute $R^2 = 0.162$, $p = 0.028$; units per 100 heartbeats $R^2 = 0.170$, $p = 0.024$). This was mirrored by positive correlations between all four MSNA measures and echo-types 3 and 4 (burst frequency $R^2 = 0.14$, $p = 0.04$; burst incidence $R^2 = 0.17$, $p = 0.02$; units per minute $R^2 = 0.17$, $p = 0.03$; units per 100 heartbeats $R^2 = 0.17$, $p = 0.02$).

No lipid profile or insulin resistance parameters had a significant correlation with MSNA or UTC data, even when controlling for group in the correlation analysis.

4. Discussion

4.1 SNS data

The current study provides the first data on *in vivo* SNS electrical activity in relation to tendinopathy. MSNA was not elevated in individuals with Achilles tendinopathy compared to healthy controls, which suggests it is unlikely to contribute to the onset of tendon pain. The correlations between symptom duration and measures of resting MSNA suggest that SNS drive becomes prominent with persistent tendon pain. This supports data indicating that the SNS plays a role in chronic pain (Janig & Habler 2000; Reeves et al. 2004). The lack of association with VISA-A scores indicates that SNS activity has a greater role in pain chronicity than pain severity.

These findings are important and likely represent chronic CNS mediated sympathetic drive being increased with longer durations of pain (Burton, Fazalbhoy 2016). This is likely due to evidence that group III and IV muscle afferent fibres project neurons to the rostral ventrolateral medulla, which is primarily involved in the baroreflex/vasoconstrictor reflex effecting sympathetic outflow (Burton, Fazalbhoy 2016). However, as it is known that tendon pain is not explained well by either CNS or peripheral nociceptive mechanisms, it is important to analyse these SNS findings in isolation as the SNS may provide a link between these two systems (Schlereth & Birklein 2008; Torebjork, Wahren 1995).

With regards to the available literature on the SNS in tendon pain, the increased chronic sympathetic drive found in the current study is therefore transmitted to additional sympathetic nerves in the paratendinous tissue (Bjur, Danielson 2008; Danielson, Alfredson 2007; Ljung, Forsgren 1999; Zeisig, Ljung 2009), although this finding is not ubiquitous

(Lian, Dahl 2006). This may also provide a mechanism for the population of abnormal tenocytes self-producing catecholamines in painful tendons (Bjur, Danielson 2008; Danielson, Alfredson 2007; Tosounidis, Hadjileontis 2013; Zeisig, Ljung 2009).

Clinically this may provide a reason why tendinopathy can become recalcitrant to treatment (Buchbinder et al. 2011; Rio, Moseley 2014). Recent treatments for recalcitrant Achilles tendinopathy are based on arthroscopically disrupting connections/adhesions between the ventral aspect of the Achilles tendon and Kager's fat pad (Alfredson et al. 2007; Danielson et al. 2008) tissue that is intimately related to tendinopathy (Ward et al. 2016) and where sympathetic fibres are abundant in tendon pain (Alfredson & Ohberg 2005; Andersson et al. 2007; Sasaki et al. 2013). Therefore, this treatment likely denervates these sympathetic fibres and diminishes the sympathetic drive to the tendon. Reducing the overactive sympathetic drive to the tendon may contribute to the success of this technique, although this at present is speculative (Alfredson & Ohberg 2005; Danielson, Alfredson 2007; Sasaki, Ohki 2013). Therefore, interventions that reduce SNS firing or block adrenergic receptors may be worthwhile research targets for individuals with chronic symptoms.

As no prior data exist on the SNS response to tendon pain or loading, this dataset advances our knowledge in the area. These data perhaps support the notion of a more chronic involvement of the SNS in tendinopathy rather than the reflexes caused by acute pain, as the SNS does not change acutely following the induction of pain in both painful and asymptomatic tendons. This is in keeping with other studies showing little MSNA change in response to certain pain stimuli (Reeves, Graff-Radford 2004).

4.2 Metabolic factors

This is the first dataset examining direct and *in vivo* measures of insulin resistance in tendinopathy. Fasting glucose levels tended to be elevated in people with tendinopathy. The difference, albeit small, in fasting glucose is consistent with other findings from diabetic (Ardic et al. 2003; Aydeniz et al. 2008) and normoglycaemic (Longo et al. 2009) participants. Furthermore, a correlation with VISA-A may indicate that fasting glucose levels relate to the severity of tendon pain.

There was no evidence to support insulin resistance as a contributor to Achilles tendinopathy. A possible explanation for the contrast with earlier studies (Ardic, Soyupek 2003; Aydeniz, Gursoy 2008; Ranger, Wong 2015) is that none of the participants in the current study had overt metabolic dysfunction. As such, there was little variability of metabolic profiles among participants. These data are, however, consistent with a portion of literature that suggests that insulin resistance does not play a significant role in tendinopathy (Altinel et al. 2007; Alvarez-Nemegyei 2007).

4.3 UTC data

UTC findings from this study showed no difference between cases and controls in tendon structure. While this is not in agreement with van Schie et al 2010 our investigation was performed in younger subjects with perhaps less time to develop significant structural change that could be detected on UTC.

The UTC findings with regards to MSNA are not conclusive as differing conclusions were reached after excluding an outlier; further investigation is warranted. If the SNS contributes

to poorer tendon structure, then UTC scanning may provide a sign of the SNS drive and chronicity of painful tendons. The discussion of this topic must be considered speculative at present, however, due to the presence of this unexplained outlier.

Finally, the lack of any correlation between metabolic measures and UTC data indicates that metabolic factors are not well associated with tendon structure. This may also be because insulin resistance only influences tendon structure when it is at an extreme level such as in those patients with T2DM (Ardic, Soyupek 2003; Aydeniz, Gursay 2008; Giacomozzi et al. 2005).

4.4 Limitations

This is the first study to examine the relationship of the *in vivo* measurements of SNS and insulin resistance in human participants with tendinopathy. However, this study design cannot comment on the temporal relationship between these factors, and therefore we must remember that correlations do not imply causation. Prospective studies are required to determine whether SNS drive increases with tendon pain chronicity.

Key limitations also arose from the sample used in this study. As differences in SNS activity were likely to be small, the small sample size may have been inadequate in uncovering these differences. Furthermore, while resting MSNA is most likely similar in both legs we cannot discount that response to tendon squeeze changes in the contralateral limb may not have provided a clear indication of the acute response in the affected limb. Additionally differing responses to pain has been shown to be multifactorial, appearing to be independent of many measurable physiological parameters (Fazalbhoy, Birznieks 2012; Kobuch et al. 2015; Schobel, Ringkamp 1996).

Moreover, the lack of pathological insulin resistance in participants may mean metabolic data were in too narrow a range to uncover any association with tendon pain or structure. Furthermore, the lack of difference in UTC data between groups may be indicative of those with tendinopathy who had relatively short pain durations. Therefore future studies should include larger sample sizes with more chronic tendinopathy in an insulin resistant population in order to further investigate the role of the SNS and insulin resistance in tendinopathy.

4.5 Perspective

The results suggest that increased SNS activity is associated with tendon pain duration. This is a significant finding building on data supporting anatomical studies on chronically painful tendons (Bjur, Danielson 2008; Danielson, Alfredson 2007; Tosounidis, Hadjileontis 2013; Zeisig, Ljung 2009). Additionally, fasting glucose was higher in those with tendon pain and was correlated with severity as measured with the VISA-A. While this study has a number of limitations, it has paved the way for larger, prospective studies to be conducted on the relationship between the SNS, metabolic conditions and tendinopathy. This study is therefore a key step in better comprehending this relationship, which may lead to a new pathophysiological understanding of tendinopathy. It also provides a rationale for investigating treatments that modulate SNS firing or block adrenergic receptors to treat chronic tendon pain.

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ACHILLES PAIN DURATION ASSOCIATED WITH MSNA

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Burst incidence

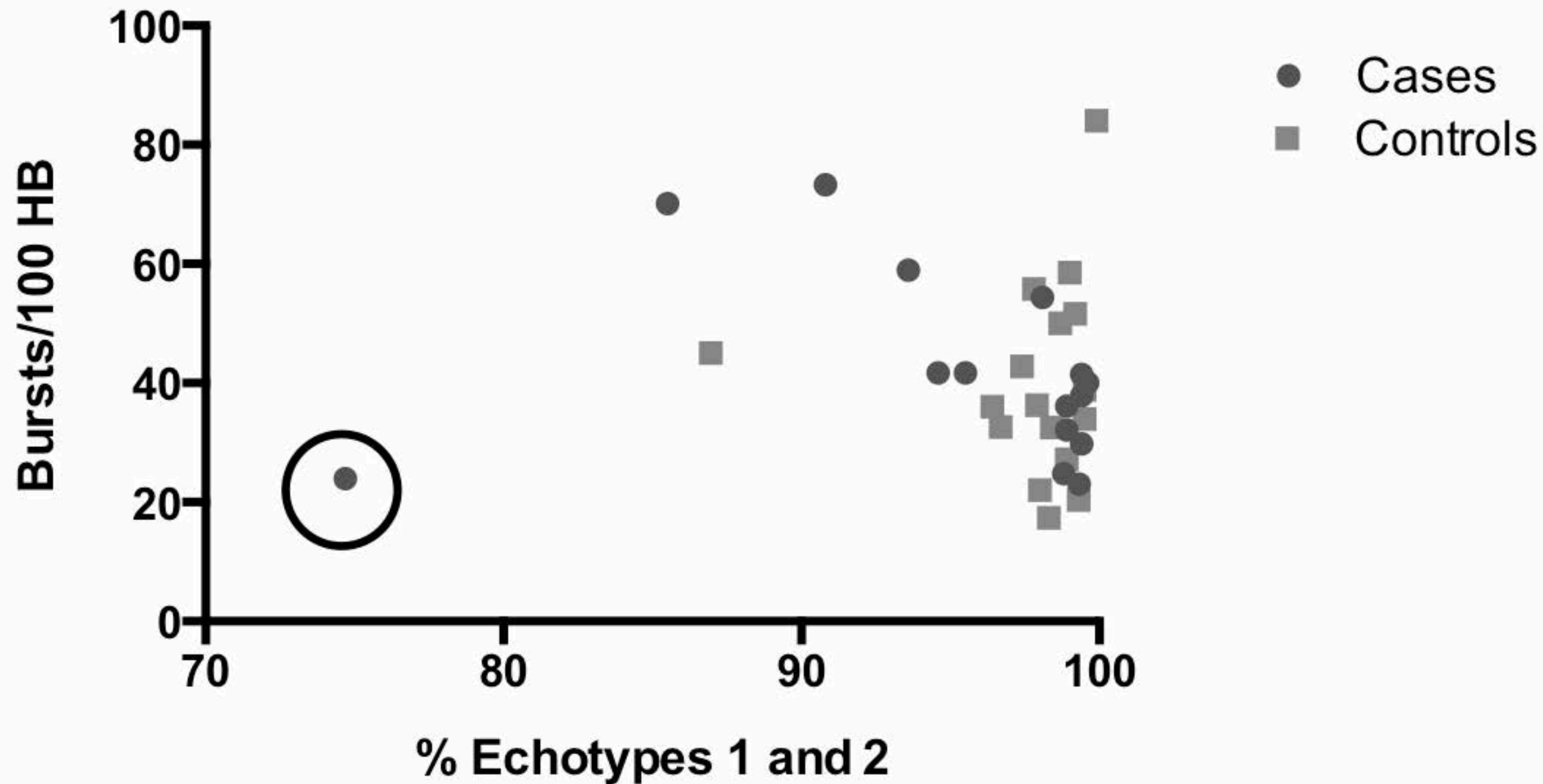


Figure legends

Figure 1: Correlation analysis of resting MSNA in relation to UTC data (% echotypes 1 and 2)

Spearman correlation: $R = -0.187$, $R^2 = 0.035$, $p = 0.305$; Adjusting for group: $R = -0.113$, $R^2 = 0.013$, $p = 0.544$. Red circle = outlying data point. $n = 15$ for cases, $n = 17$ for controls, $n = 32$ overall.

Table 1: Eligibility criteria

	Cases	Controls
Inclusion criteria	• Be over 18 years of age • Symptoms and examination findings consistent with clinical Achilles tendinopathy – focal pain, related to tendon loading (e.g. running) • Symptoms for at least 1 month • Symptoms within 2 months	• Be over 18 years of age
Exclusion criteria		• Previous or current tendinopathy
	• Been administered intravenous or injected corticosteroids in past 12 months • Been administered fluoroquinolone antibiotics in past 12 months • Previous Achilles rupture or surgery • Diagnosis of a systemic inflammatory disease • Diagnosis of cancer within the last 24 months • On regular medications which can effect SNS (e.g. selective serotonin reuptake inhibitors)	

Table 2: Demographic, anthropometric and questionnaire data

Categorical variable		Case (n=15)	Control (n=20)	p-value
Sex	Female	6	7	0.762
	Male	9	13	
Alcohol	Never	2	2	0.441
	< 1/week	5	11	
	> 1/week	8	7	
Smoking	Never	14	18	0.727
	Past	1	2	
	Current	0	0	
Illicit drugs	Never	12	18	0.468
	Past	2	2	
	Current	1	0	
Prescribed medications	Oral contraceptive pill	1	3	0.719
	Others not effecting nervous system	3	3	
	None	11	14	
Continuous variable		Median, IQR	Median, IQR	p-value
Age (years)		30.0, 20.0	24.50, 7.75	0.131
BMI (kg/m²)		23.0, 4.74	23.0, 3.55	0.755
Waist/hip ratio		0.808, 0.10	0.824, 0.08	0.882
VISA-A		67.0, 21.0	100, 0.00	< 0.001*
Running years		10.0, 26.0	10.0, 11.5	0.320

Running km/week	5.00, 15.0	7.50, 14.8	0.633
Systolic blood pressure (mmHg)	112, 9.34	117, 16.8	0.730
Diastolic blood pressure (mmHg)	68.7, 13.3	67.5, 12.8	0.227

*Statistically significant

IQR = interquartile range

Table 3: Tendon pain characteristics in case group (n = 15)

Duration		Median = 24 months Range = 1 – 396 months
Consulted professional	Yes	8 (53.3%)
	No	7 (46.7%)
Site	Mid-portion bilateral	6 (40.0%)
	Mid-portion unilateral	6 (40.0%)
	Insertional bilateral	2 (13.3%)
	Insertional unilateral	1 (6.7%)
Experienced symptoms within last week	Yes	10 (66.7%)
	No	5 (33.3%)

Table 4: Resting MSNA data between groups

	Case (mean $\pm$ SD) n=15	Control (mean $\pm$ SD) n=17	p-value
Burst frequency (bursts/min)	24.3 $\pm$ 9.02	22.5 $\pm$ 9.04	0.563
Burst incidence (bursts/100 HB)	42.0 $\pm$ 15.8	40.3 $\pm$ 16.5	0.774
Units/min	1069 $\pm$ 382	926 $\pm$ 334	0.264
Units/100 HB	1855 $\pm$ 708	1665 $\pm$ 644	0.433
Heart rate	58.8 $\pm$ 8.46	56.9 $\pm$ 7.62	0.526

SD = Standard deviation

Table 5: Metabolic factors between groups

	Case (median, IQR) n=15	Control (median, IQR) n=20	p-value
Fasting glucose mmol/L	4.80, 0.70	4.51, 0.38	0.017
Maximum glucose mmol/L	6.40, 2.50	5.25, 2.83	0.610
Fasting insulin mIU/L	4.00, 6.00	4.00, 4.75	0.610
Matsuda index	9.40, 21.5	19.4, 19.7	0.227
Total cholesterol (TC) mmol/L	4.50, 1.70	4.65, 1.00	0.831
Triglycerides mmol/L	0.90, 1.20	1.00, 0.80	0.908
HDL-C mmol/L	1.40, 0.50	1.45, 0.48	0.382
LDL-C mmol/L	2.80, 1.60	2.65, 1.08	0.542
TC/HDL-C	3.71, 1.22	2.97, 1.32	0.227
LDL-C/HDL-C	2.07, 0.99	1.75, 1.12	0.364
TG/HDL-C	0.73, 1.06	0.70, 0.55	0.610