

# **ORIGINAL ARTICLE**

# A comparative study between low and high intensity percutaneous needle electrolysis in patients with patellar tendinopathy: a structural and functional analysis

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**KEYWORDS** Tendinopathy; Patellar tendon; Ultrasound-guided percutaneous needle electrolysis

## Abstract

Objective: The aim of this study is to compare two different ultrasound-guided percutaneous needle electrolysis modalities applied at the same dose: low intensity/long time period application versus high intensity/short time period application, in subjects with patellar tendinopathy. Material and method: An experimental comparative study involving 13 subjects diagnosed with patellar tendinopathy. Subjects were divided into two different groups. Percutaneous electrolysis was applied to 17 tendons. Group 1 was treated for 6 weeks, and received 1 weekly session consisting of 3 interventions at 3 mA during 3 seconds. Group 2 was also treated for 6 weeks, however the weekly sessions consisted of 3 interventions at 0.3 mA during 30 seconds. Morphological changes were evaluated using ultrasound (echogenicity was evaluated based on their grey scale, vascularization and examinations performed at 0.5 and 1 cm below the apex of the patella). The functional changes were evaluated using the VISA-P scale. The VAS scale was used to compare both the pain during application and the post-needling pain for both treatment methods.

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Results: All subjects in group 2 showed improvements in their VISA-P scores. However, only subjects in group 1 presented structural changes in the tendon area 0.5 cm below the apex of the patella, as well as similar changes in the VISA-P scores. There were no variations in perceived pain between the two methods.

Conclusions: Only high intensity/short time period applications of percutaneous electrolysis generate structural changes compatible with tendon regeneration. However, both modalities cause similar pain during application and post-needling.

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PALABRAS CLAVE Tendinopatía; Tendón rotuliano; Electrolisis percutánea ecoguiada

#### Estudio comparativo de la electrolisis percutánea a baja y alta intensidad en la tendinopatía rotuliana. Análisis funcional y estructural

#### Resumen

*Objetivos*: Comparar la aplicación de dos modalidades de electrolisis percutánea con la misma dosis (intensidades bajas y tiempos prolongados *versus* intensidades altas con tiempos cortos), en sujetos con tendinopatía rotuliana.

*Material y métodos*: Estudio experimental comparativo de 13 sujetos diagnosticados con tendinopatía rotuliana. Los sujetos se dividieron en dos grupos. Se aplicó electrolisis percutánea a un total de 17 tendones. El grupo 1 fue sometido, durante 6 semanas, a una aplicación semanal de tres intervenciones, de 3 mA, durante 3 segundos. El grupo 2 fue sometido, durante 6 semanas, a una aplicación semanal de tres intervenciones, de 0,3 mA, durante 30 segundos. Se analizaron los cambios morfológicos a través de la ecografía (ecogenicidad mediante la escala de grises, la vascularización y el área a 0,5 y 1 cm del pico de rótula). Con el fin de evaluar los cambios funcionales se utilizó la escala VISA-P. Para comparar el dolor de aplicación y post-punción entre ambos grupos se recurrió a la escala EVA.

*Resultados*: En el grupo 2 todos los sujetos experimentaron mejorías en su calificación a través de la VISA-P, pero sólo los sujetos del grupo 1 presentaron, además de cambios similares en la VISA-P, cambios estructurales en el área del tendón a 0,5 cm del pico de rótula. No hubo variaciones de dolor entre ambas modalidades.

*Conclusiones:* Sólo las aplicaciones con intensidades altas y tiempos cortos generan cambios estructurales compatibles con regeneración tendinosa. Sin embargo, ambas modalidades provocan dolor similar en la aplicación y post-punción.

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#### Introduction

Patellar tendinopathy is an overuse syndrome that mainly affects the tendon at its insertion on the inferior pole of the patella<sup>1</sup>. This is a highly incapacitating condition for athletes, which is highly associated with disciplines that generate tendon loading (14% of all elite athletes). The prevalence rates for patellar tendinopathy in sports such as volleyball (45%) and basketball (32%) are at the top of the statistics in adult athletes<sup>2</sup>, although this pathology is not exclusively related with age<sup>3</sup>.

Regarding physiopathology, although there are studies that demonstrate the absence of inflammatory cells in tendinopathies and the presence of degenerative changes<sup>4</sup>, others have detected the presence of inflammatory mediators<sup>5</sup>. The debate regarding the importance of inflammation *versus* degeneration is still applicable<sup>6</sup>, however it is now accepted that both processes may, at times, occur simultaneously.

At present, despite the magnitude of the problem regarding patellar tendinopathy and its clinical importance, there is a striking lack of consensus regarding the ideal therapeutic measures for the treatment of patellar tendinopathy<sup>7</sup>. Nonetheless, multiple systematic reviews<sup>8,9</sup> and randomized clinical trials (RCTs)<sup>10,11</sup> have been published regarding the management of patellar tendinopathies using different pharmacological measures, such as non-steroid anti-inflammatory drugs (NSAIDs)<sup>8</sup> and infiltrations with corticosteroids<sup>9</sup>; plus other therapeutic options such as platelet rich plasma (PRP)<sup>10</sup> or, even, surgical measures<sup>11</sup>. Despite this, the problem lies in the lack of scientific evidence regarding the application of many treatments<sup>1</sup>, even though many of these are still being applied in practice. In this sense, physiotherapy has developed new techniques for the treatment of patients with patellar tendinopathy and which have demonstrated their effectiveness, such as the case of percutaneous needle electrolysis (PNE)<sup>12,13</sup>.

PNE is a technique of invasive physiotherapy consisting of the application of an ultrasound-guided high intensity galvanic current through the skin using a dry needling needle. This produces both an analgesic effect in the musculoskeletal soft tissue and a local inflammatory process, which enables phagocytosis and the repair of the affected tissue<sup>14</sup>. These effects justify the use of PNE in the treatment of patellar tendinopathy, which has been the subject of various research publications, both *in vivo* using human models, as well as *in vitro* on animal models<sup>12,15,16</sup>.

A galvanic current is a continuous current that has been used in medicine with a number of therapeutic aims. The continuous current refers to the continuous flow of electric charge via a current conductor between two points with different potentials, which does not change direction over time. In contrast to alternating currents, the electrical charges always flow in the same direction. The electrical charge is directly proportional to the relation between the time and the intensity, and thus the Dose = Electrical charge (measured in coulomb) = Time (measured in seconds)  $\times$  intensity (measured in amperes).

Since the appearance of new modalities for applying low intensity PNE, which is defined as "unpainful electrolysis", it is necessary to demonstrate whether or not there are differences in the effectiveness of this technique when applied at low intensities over the same dose of electrolysis.

Thus, the main aim of this study was to compare two application modalities of PNE with the same dose, in subjects with patellar tendinopathy regarding pain, function and structural changes at the level of the tissue receiving therapy. Prior to this, an instrumental analysis was performed on various devices used for the application of percutaneous galvanic currents in order to analyse the characteristics of the same and to test the calibration of each of these.

# Material and methods

### Study design

A prospective, experimental study was performed between March and May of 2016 involving subjects referred to the physiotherapy centre "Centro de fisioterapia, readaptación y entrenamiento Fix&Fit" (Las Palmas de Gran Canaria). This was a comparative study based on two application modalities of PNE: lower intensities and longer time periods versus higher intensities and shorter times.

All subjects fulfilled the following inclusion criteria: aged between 18 and 45 years, with a medical diagnosis of proximal patellar tendinopathy of more than three months' evolution and not having previously received treatment with PNE, or never successfully completing the same. The exclusion criteria were: having received treatment via corticoid infiltration in the previous 3 months, having received surgical techniques in the same knee or any contraindications for the application of PNE.

The sample of subjects that participated in the study was divided into two groups according to the register numbers: 1 and 2. Group 1 received high intensity PNE during short periods, and group 2 received low intensity PNE during prolonged time periods. As a result of the division of both groups and the bilateral affectation of some subjects, the groups were divided in a non-equal manner: 7 subjects in group 1 and 10 subjects in group 2. All subjects were blinded and duly informed of the study and the treatment they were to receive. The Research Ethics Committee of the University CEU San Pablo approved the study, which meets all the principles established within the Helsinki declaration. All subjects signed the corresponding informed consent form in order to participate in this study.

#### Variables analysed

The structure of the patellar tendon was analysed, as well as subjects' function and pain. For the assessment of structure, a morphological and structural analysis of the patellar tendon was performed using musculoskeletal ultrasound, following the parameters standardised by the European Society of Musculoskeletal Radiology<sup>17</sup>. With the patient in the supine position and with the knee at 30° flexion, a B mode study was performed with longitudinal and transverse sections at 0.5 and 1 cm from the inferior pole of the patella, in order to assess tendon thickness, changes in echogenicity, the presence of calcifications, etc. Also, the presence of neovascularization was analysed using Power Doppler Imaging (PDI). The echogenicity of the tendon was evaluated using the scale described by Maillaras et al.<sup>18</sup> and the vascularization was assessed using the classification by Del Buono et al.<sup>19</sup>. All uncompressed images and videos were stored for subsequent analysis.

The ultrasound examination was always performed by the same observer, who had 2 years' experience using ultrasound and who performed a prior test-retest study. A LOGIC F6 device was used by General Electric with a L6-12-RS probe and a frequency range of 4-13 MHz. The transmission gel used was Aquasonic contact gel. Also, a minimal amount of pressure was used in order to avoid deforming the tissue under study. These data were registered prior treatment (at baseline), after the third session, and at the end of the study (at 0,3 and 6 weeks).

In order to perform the functional assessment, a Spanish adapted version of the VISA-P (Victorian Institute of Sport Assessment)<sup>20</sup> was used, the VISA-P-Sp<sup>21</sup>, to register the individual's functional changes after the application of PNE.

The Visual Analogue Scale (VAS) was used for the assessment of pain. The values of treatment application pain were registered immediately post intervention. In order to evaluate the post-needling pain, subjects were asked to memorize their level of pain 48 hours after the intervention and these values were registered at the next session.

#### Physiotherapy interventions

Both groups were treated by the same physiotherapist, who had 7 years' experience in the performance of the PNE technique. Percutaneous electrolysis treatment was applied in an isolated fashion, at a rate of one session per week during a 6-week period. Treatment was applied with ultrasound guidance and under conditions of asepsis, over the inferior pole of the patella, using a distal-proximal approach, on the long axis, with a  $45^{\circ}$  orientation and prior control of the needle guidance through the ultrasound window using PDI according to the methods and protocol described by Valera and Minaya<sup>22</sup> (fig. 1).

In group 1 (high intensity-short time periods) 3 impacts at 3 mA and lasting 3 seconds were performed during each session over the deep tendon fibres at the level of the enthesis on the lower pole of the patella (fig. 1). In group 2 (low intensity-prolonged time periods) 3 impacts lasting 30 seconds were performed at 0.3 mA over the same treatment area<sup>12,13,22,23</sup>. In both groups, the dose was 9 C (coulombs) per impact. We did not use cryotherapy, not supportive bandages after the intervention, nor were anti-inflammatory drugs taken for a minimum of 72 hours.

#### Instrumental analysis

Previously, an intensity calculation was performed using an ammeter on several galvanic current devices used for PNE. Thus, we analysed the Physio Invasiva® device by PRIM Fisioterapia (2016), the XMOmega model EPI® device, by Genius Chip Limited, and an EPTE® SYSTEM device by IONCLINICS (2014). All devices had the CE marking for the percutaneous application of galvanic currents. In order to perform the measurements, an experiment with a human



**Figure 1** Intervention with percutaneous electrolysis in the deep fascicle of the patellar tendon in the immediate surroundings of the inferior pole of the patella.

model was designed in which an approach was performed on the brachioradialis muscle, at a depth of 3 cm, using the same type of needles as those used for the PE interventions over the patellar tendon. Three interventions were performed, lasting 3 seconds and at 4 different intensities: 0.3 mA (the minimum for Physio Invasiva®), 1 mA, 2 mA (maximal compatible intensity across all devices) and 3 mA. Although the 3 mA intensity was not adjustable on all devices, this intensity was included as it is frequently used in high intensity treatments. The measurements were performed with an ammeter by the FLUKE 175 TRUE RMS MUL-TIMETER brand, which has a sensitivity of 0.01 mA for galvanic currents.

#### Statistical analysis

For the introduction, management and analysis of data, MatLab software was employed. The Mann-Whitney U test for non-parametric variables was applied, as this is the most indicated test due to the size of the sample and because it is more restrictive for rejecting the formulated hypothesis, thus decreasing the chances of a false positive. The significance level was set at p< 0.05, and in the case of an error when rejecting the null hypothesis, the level of significance increased until the maximal value of p< 0.2.

### Results

Thirteen subjects were included in this study, with 17 tendon samples, as 4 subjects had bilateral affectations. Of these, 6 tendons were on the right side and 11 were on the left. Of all subjects 5 were women (38%) and 8 were men (62%), with a mean age of 33 years (SD: 7.7) (table 1).

## Tendon structure

The initial vascularisation assessment for group 2 revealed a classification of 3 (SD: 1.10) and the final value was 2.6 (SD: 0.84). The variation of the means obtained a value of -0.3 (SD: 0.26). In the case of group 1, the initial value was 3.29 (SD: 1.29) and the final value was 2 (SD: 0.82). The variation was -1.29 (SD: 0,30). In the case of the greyscale findings, the initial value for group 2 was 2 (SD: 0.52) and the final value was 2.3 (SD: 0,48). The difference was 0.3 (SD: 0.26). In group 1, the initial mean was 3.29 (SD: 1.11) whereas the final mean was 2 (SD: 0,82). The difference equalled -1.29 (SD: 0.30).

The mean of the tendon areas at 1 cm at the onset of the study in group 2 was  $1.51 \text{ cm}^2$  (SD: 0.64) and upon completion of the study this was  $1.52 \text{ cm}^2$  (0.61). The result of the difference was 0.01 cm<sup>2</sup> (SD: 0.03). In the case of group 1, initially the mean was  $1.30 \text{ cm}^2$  (SD: 0.31) and at the end of the study this was  $1.27 \text{ cm}^2$  (SD: 0.35). The difference was  $-0.02 \text{ cm}^2$  (SD: 0.05). The areas at 0.5 cm from the lower pole of the patella in group 2 produced an initial mean of  $1.60 \text{ cm}^2$  (SD: 0.50), whereas at the end this was  $1.63 \text{ cm}^2$  (SD: 51). The difference was  $0.03 \text{ cm}^2$  (SD: 0.02). In group 1,

 Table 1
 Description of the sociodemographic characteristics of the sample

Group	Age	Sex	Height	Weight	Level of Activity	Laterality	Evolution (months)
2	33	F	156	59	Sedentary	Bilateral	3
1	42	Μ	180	66	Amateur	Left	12
2	36	Μ	180	76	Amateur	Left	48
1	36	Μ	180	78	Amateur	Left	72
2	30	Μ	175	105	Active	Right	24
1	31	F	161	52	Active	Left	8
2	38	Μ	176	80	Active	Right	120
1	39	Μ	168	64	Active	Bilateral	216
2	44	Μ	178	79	Active	Left	42
1	37	Μ	170	72	Amateur	Left	3
2	18	F	170	57	Amateur	Bilateral	18
1	21	F	160	56	Active	Left	3
2	27	F	157	55	Active	Bilateral	120

the mean initial values were 1.49 cm<sup>2</sup> (SD: 0.66) whereas the final values were 1.33 cm<sup>2</sup> (SD: 0.37); with a difference of -0.15 cm<sup>2</sup> (SD: 0.30) (fig. 2).

In order to evaluate the effects of treatment on structure, only the measurements of the areas at 0.5 and 1 cm were described as response variables, as the application of both modalities of PNE did not generate sensitive changes on the vascularization and greyscale values.

For the analysis of the area at 0.5 cm distance, the nonparametric Mann-Whitney U test was used, obtaining a pvalue of 0.025 (p< 0,05), and therefore the null hypothesis was rejected at a significance level of 5%. On the other hand, using the same test, the analysis of the area at 1 cm concluded that the p-value was 0.2803 (p> 0,05), both at a 5% level of significance as well as 20%; and therefore the null hypothesis could not be rejected for this response variable.

#### Functional analysis (VISA-P)

The mean baseline value of the VISA-P in subjects in group 2 was 57.4 (SD: 18.81) and the mean value in the last assessment was 72.9 (SD: 13.71). This difference reveals a mean variation of 15.5 (SD: 5.10). The mean baseline value of subjects in group 1 was 71.14 (SD: 5.34) and the mean value in the final assessment was 88.96 (SD: 8.67). The difference reveals a mean variation of 17.71 (SD: 3.33). According to these findings, after 8 weeks of treatment (six applications of PNE), there were no statistically significant differences between both groups (fig. 3).

# Relation between pain during treatment application and current intensity

The mean of the VAS assessments for pain at the time of application in group 2 was 4.72 (SD: 2.33) whereas for group 1, this was 5.57 (SD: 1.77) (fig. 4).

Considering the response variable as the average of the obtained values, the result of the non-parametric Mann-Whitney U test provided a p-value of 0.7136 (p> 0.05). No differences were therefore found between the perceived pain in response to treatments applied at the different intensities.



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# Relation between post-puncture pain and current intensity

The mean results for the VAS measures for post-needling pain in group 2 was 2.25 (SD: 2.39) and those for group 1 were 1.71 (SD: 2.02) (fig. 4), with a p-value result of 0.1926. Consequently, the null hypothesis cannot be rejected at 5% significance, although the opposite is true for 20% significance.

# Instrumental analysis

Table 2 describes the calibration data and the precise settings of intensity as a result of the instrumental analysis. Regarding the characteristics of the devices, we observed that the Physio Invasiva® device enables intensity variations of 0.1 mA, from 0.3 to 2 mA, and from 0.5 mA, starting at 2 mA and reaching a maximum of 15 mA. The EPI® device enables intensity variations from 0.1 mA up to 1 mA and, from there on, it increases in ranges of 1 mA until a maximum of 15 mA. On the other hand, the EPTE® device enables intensity increases of 0.01 mA from 0.05 up to 2.5 mA. It is important to note that the EPTE® electrostimulation device is programmed in a way that, in order to generate a dose of



**Figure 2** Variation of the area of the tendon at 0.5 cm distance from the patella.



Device		Calibratio	Precision		
	0.3 mA	1 mA	2 mA	3 mA	
Physio Invasiva®	0.36	1.02	2.03	3.07	$\Delta$ 0.1 mA (from 0.3 to 2 mA)
					Δ 0.5 mA (>2 mA)
<b>EPTE</b> <sup>®</sup>	0.32	1.02	2.01	-	Δ 0.01 mA
EPI®	0.43	1.3	2.04	3.03	$\Delta$ 0.1 mA (from 0.1 to 1 mA)
					Δ 1 mA (>1 mA)

1 mA during 5 seconds, the intensity increases progressively with a ramp of approximately 15 seconds (measured with a Casio brand chronometer), subsequently remaining for 5 seconds at the preselected intensity (table 2).

# Discussion

Presently there are several existing publications on the use of PNE in patellar tendinopathy<sup>12,13,23</sup> which report improvements in function and pain. However, no publications have demonstrated morphological changes objectively visualised under ultrasound after the application of PNE, although this is the case of the lateral epicondylalgia<sup>24</sup>. In the experiments on patellar tendinopathy by Abat et al. (2014 and 2015), the authors report ultrasound changes in a patient based entirely on before and after treatment images, without describing morphological changes in the results section. In the case of Valera et al. (2010), significant changes were not found on the ultrasound examination after six weeks of treatment, however these authors hypothesize in the discussion that the structural changes may in fact be present in the short term, however visualization of the same may be limited due to the technology of the time. Regarding the low intensities, there is scarce literature on this subject<sup>25-27</sup>, including a study of three cases of patellar tendinopathy<sup>26</sup>. No studies have compared the use of high and low intensities during PNE, and this is the first research paper to do so.

In this study, changes in vascularisation were not registered, not even in the tendon area at 1 cm from the inferior pole of the patella. The relevant changes occurred at an area of 0.5 cm from the pole of the patella. This may be due to the fact that the single intervention performed was at the level of the deep enthesis on the apex of the patella. This may explain why that the changes were observed in the section closest to the treated area. Interventions were not performed over the neovessels, nor in the deep interphase of the tendon with the Hoffa fat. It is possible that if this type of approach had been performed, changes would have been found in the vascularisation and the tendon width at 1 cm from the apex of the patella.

Similar to other studies on PNE in which the VISA P-Sp has been used<sup>12,13,15,23</sup>, the subjects in each group considerably increased their score on the scale. The fact that, on a functional level, there is no difference between both groups may be related with the analgesic effect of the galvanic current. However, the morphological changes identified can only be related with the regeneration of the tendon caused by the high intensities. Despite using the same dose, the results in the tissue were different. It is likely that the applications at low intensities and for longer time periods generate physiological adaptation mechanisms in the organism that provoke a smaller inflammatory response and a limited proliferation of the corresponding tissue.

The lack of relation between the intensity parameter and the pain scores during treatment application and post-needling means applications with low intensities sought to avoid pain are not justified. Pain levels were the same for both intensity modalities studied and, furthermore, the improvement is less at the low intensities, which is limited to functional parameters. The reason the low intensity modality is not pain free nor less painful then the high intensity application may be due to the insertion of the needle and the longer application time of PNE, which causes a greater perception of pain for the patient. In order to be able to associate the painful sensation of both modalities to the needling or to the exposure to a current, other experimental models are needed, for example, in which several VAS are administered to subjects in order to register all the possible painful sensations.

Considering our results, we can infer that the appearance of pain during the application of electrolysis is necessary in order to activate the nervous system processes which contribute towards inducing the activation of complex endogenous mechanisms for pain modulation<sup>28</sup> and fight or flight responses<sup>29</sup>, although at the same time, too much pain may be an unwanted side effect.

In this study, only 3 subjects presented a VISA-P assessment below 50 points. However, 9 of the subjects (70%) modified their physical activity, finding alternatives that were less painful and aggressive towards the patellar tendon; therefore, when asking them about their relation between physical activity and pain, the outcome was quite high. For a high percentage of the cases under study (77%), subjects obtained the maximum value in some of the options in question 8. They were therefore able to perform an adapted sports activity during 30, 60 or 90 minutes or longer, as well as with or without, pain (10, 20 and 30 points on the VISA-P, respectively). In a maximal total score, there was only a difference of 6 points (6% of the total) between performing a modified activity and performing a competitive activity at the same level, or greater, than that of before the appearance of the symptoms; and 3 points difference (3% of the total) compared to performing the same physical activity at a lower level than before the appearance of symptoms. However, to be able to perform a

physical activity during more than 60/90 minutes, even when this is modified or generates pain, adds up to 20 or 30% of the total score. This is related to errors in the correlated items 7 and 8, described by Hernández-Sánchez, and dependent on a second factor<sup>21</sup>.

Considering the above, the performance of further research on the sensitivity of the VISA-P scale towards the functional alterations of subjects who have modified their physical activity seems necessary, perhaps even the performance of a modified version of the same.

Regarding the results of the instrumental analysis, it is important to highlight those of the EPTE® electro stimulator. Despite the fact that this device enables the sequential performance of a greater number of intensity adjustments (0.01 mA), in the calculation of the dose the ramp for increasing intensity was not considered. This means it is impossible to know the exact dose and be able to compare the doses applied between this and other devices. It would be necessary to perform a study with more complex devices (oscilometers), which enable the precise calculation of the curve of the ramp in order to have a greater certainty of the dose applied.

## **Study limitations**

The small sample size, lack of randomization and asymmetry of the groups means the hypothesis test has a limited reliability. Regarding the assignment of the subject groups, this method was used in order to guarantee, as much as possible, that both groups were comparable in number. Despite this, the fact that the tendinopathy was present bilaterally in some subjects meant that, although the groups are comparable, they are not exactly equal in number. On the other hand, the data collection was performed over a relatively short period, therefore it would be necessary to perform a longer follow-up of subjects in order to verify that the changes are maintained in time or whether, in contrast, a greater rate of recurrences occurs in group 2 (having not registered morphological changes).

The ultrasound device used in this study was another limitation because as it is a medium range device, it probably does not offer the maximal necessary resolution in order to identify more substantial changes in the tissue. It would be interesting to repeat this study with more advanced ultrasound devices and even include a study using sonoelastography, as this increases the sensitivity for the diagnosis of tendinopathy<sup>30</sup>. Despite this, considering that the structural changes were found in the section closest to the treated area, this study justifies the need for performing an ultrasound-guided approach in order to precisely locate the target tissue and optimize the effectiveness with the greatest security measures possible<sup>31</sup>.

In conclusion, although the functional results are the same after a six-week application, the effects of the PNE application at low and high intensities produce different effects on the morphological level. The application modality using high intensities generates greater changes in the area of the stimulated tendon, associated with regeneration of the same. However, both application modalities provoke a similar level of pain, both during application as well as postneedling.

# **Conflicts of interest**

The authors declare no conflicts of interest.

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