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Analysis of Achilles tendon vascularity with second generation Contrast-Enhanced Ultrasound (CEUS)

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Review

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ABSTRACT

Purpose: To compare morphological and power Doppler features of the Achilles tendon and Contrast-Enhanced Ultrasound (CEUS) behaviour between asymptomatic athletes and athletes who had undergone surgery for repair of a previous Achilles tendon rupture.

Methods: 24 athletes were divided in two groups (A and B). Group A included 14 patients with a median age of 32 years (range 27 to 47 years) who had undergone surgical repair for unilateral Achilles tendon rupture. Group B (control group) included 10 subjects with a median of 34 years (range 27 to 40 years) with no previous or present history of tendinopathy. All patients were evaluated with ultrasound (US), power-Doppler and CEUS with second-generation contrast agent. We studied the uninjured Achilles tendon in athletes who had suffered a previous rupture (Group A) and either the left or right Achilles tendon of the athletes in Group B.

Results: CEUS showed a significantly greater ability to detect a greater number of vascular spots within the tendon of Group A compared to Group B (< 0.05).

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2
3 **Conclusions:** In athletes who had suffered a tear of an Achilles
4 tendon, CEUS detected small vessels that were not identified by
5 power-Doppler US in the uninjured controlateral Achilles tendon.
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10 CEUS is useful to evaluate vascularity not detected by other imaging
11 techniques. Vascularity seems to be increased in patients who had
12 suffered from a previous rupture.
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20 INTRODUCTION

21
22 Many authors described a direct relationship between tendinopathy
23 and increased vascularisation in Achilles tendons, even though this
24 relationship is controversial [1-3]. Contrast-Enhanced Ultrasound
25 (CEUS) with second-generation contrast agent demonstrated a high
26 sensitivity to evaluate vascularity of different tissues [4-7]. Recently,
27 Rudzki and co-authors have used a contrast-enhanced ultrasound
28 method to demonstrate regional variations in the vascularity of the
29 supraspinatus tendon, with an age-dependent decrease in
30 asymptomatic individuals with intact rotator cuffs [8]. Patients who
31 suffer from an acute Achilles tendon rupture have a nearly 200-fold
32 increased risk of a contralateral tendon rupture [9] and many exhibit
33 intratendinous changes in the contralateral Achilles tendon. This
34 observational study compared the sensitivity of power Doppler US
35 and CEUS to assess the vascularisation of the Achilles tendon in two
36 different populations. The first one was composed by subject who had
37 sustained an Achilles tendon rupture and in which the contralateral
38 tendon was assessed, and the second one in which either the right or
39 left healthy tendon was studied. We wanted to verify whether CEUS
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3 has a higher sensitivity than power Doppler US in detecting
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5 vasculature and that vascularity is increased in patients who suffered
6
7 from a previous rupture.
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10 11 12 **MATERIALS AND METHODS**

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15 All the procedures described in the present study were approved by
16
17 our Institutional Review Board, and all participants gave written
18
19 informed consent to participate in the study.
20
21

22 Twenty-four amateur runners were included in the study; all athletes
23
24 were running at least three times in the week for at least 15 km. They
25
26 were divided in two groups. Group A was composed of 14 patients, 12
27
28 males and 2 females, with a median age of 32 years (range 27 to 47
29
30 years). All had undergone surgery for a unilateral Achilles tendon
31
32 rupture in the past two years from the present study. None of them had
33
34 symptoms from the controlateral tendon. Group B was composed of
35
36 10 asymptomatic subjects (7 males and 3 females, with median age of
37
38 34 years (range 27 to 40 years) with no previous tendon pathology.
39
40
41 The non affected Achilles tendon of group A subjects, and either the
42
43 left or the right Achilles tendon (selected by the subject tossing a coin
44
45 immediately before undergoing the ultrasound examination) of group
46
47 B subjects were studied with gray-scale US, power Doppler and
48
49 CEUS.
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54 All subjects were asked to abstain from sports activities for two days
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56 before the evaluation. The exams were conducted with a Technos
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58 MPX US (Esaote, Genova, Italy) by the senior radiologist with the
59
60 patient prone and the ankle at 90°; he was blinded to knowing whether

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2
3 a patient belonged to group A or B. A gray-scale US and power-
4
5 Doppler with a 13-4 MHz linear probe, with longitudinal and
6
7 transversal scans, were used to detect any structural tendon
8
9 abnormalities. Anteroposterior thickness, US-echotexture
10
11 (homogeneous / dishomogeneous), and signs of peritendinopathy were
12
13 evaluated at the middle one third and insertion of the Achilles tendon
14
15 on the calcaneus. Patients who showed clinical signs of tendinopathy
16
17 were excluded from the study. For power Doppler evaluation, the gain
18
19 was set to cancel any noise signal. CEUS was then performed using
20
21 longitudinal scans, with a linear 9-3 MHz probe and dedicated CnTi
22
23 software (Esaote, Genova, Italy). The radiologist administered a 5 ml
24
25 volume injection of second-generation contrast agent (Sonovue,
26
27 Bracco, Milano, Italy) followed by 10 ml saline solution in an
28
29 antecubital vein of the forearm of all the subjects involved in the
30
31 present study. Two videoclips of 60 seconds each were recorded,
32
33 beginning at the end of the injection of the contrast agent. The level of
34
35 contrast diffusion in the tendon was evaluated in a quantitative fashion
36
37 drawing time intensity enhancement curves, using Qontrast™
38
39 software (Bracco, Milano, Italy). The intensity of contrast diffusion in
40
41 the tendon was calculated on longitudinal tendon scan. The variables
42
43 analyzed on the curves were the following:
44
45
46 PEAK: maximum signal intensity value;
47
48 TTP (Time to Peak): time that the contrast agent takes to reach its
49
50 higher concentration;
51
52 RBV (Regional Blood Volume): blood volume proportional to the
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54 area under the curve
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3 (AUC), calculated as the integral of the same curve;
4

5 RBF (Regional Blood Flow): blood flow, calculated as the ratio
6
7 between RBV and MTT (medium transit time).
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10 Data were entered in a commercially available software. Descriptive
11
12 statistics were calculated. All analyses were performed using the SPSS
13
14 13.0 statistical software package (SPSS Science Inc., Chicago, IL). A
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16 Mann-Whitney's test for independent samples was used, considering
17
18 significant a p value < 0.05.
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27 **RESULTS**

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29 The gray-scale analysis did not show any pathological thickening at
30
31 the middle third and the region of insertion on the calcaneus site of all
32
33 the tendons evaluated. The anterior-posterior mean diameter was $4.6 \pm$
34
35 3.10 mm in group A and 4.55 ± 3.02 mm in the group B. The tendon
36
37 structure was homogeneous without any focal hypo- or hyper-echoic
38
39 lesions. We did not detect oedema in the tendon. Power Doppler exam
40
41 did not show any intra-tendinous neovascularisation in either group.
42
43
44 The results of CEUS are summarized in Tables I and II. The
45
46 enhancement curves were drawn considering the data of the second
47
48 videoclip because the presence of the contrast agent was appreciable
49
50 in all subjects only at 40-60 seconds from the injection. In two male
51
52 patients of group A, it was not possible to calculate the enhancement
53
54 curve due to movement artefacts. Group A subjects showed a values
55
56 of peak (mean 3,9; SD 0,07), TTP (mean 40,2; SD 5,1), RBV (mean
57
58 267; SD 142) significant higher than Group B subjects (peak mean
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3 2,8; SD 1,07; TTP mean 31,9; SD 5,9; RBV mean 150; SD 56,8) with
4
5 a $p < 0.0$). No difference was observed for the RBF parameter (mean
6
7 4,1; SD 1,5 in Group A and mean 3 with SD of 1,1 in Group B).
8
9

10 11 12 **DISCUSSION**

13
14
15 Midsubstance ruptures of the Achilles tendon typically occur in 30–50
16
17 year old male recreational athletes [9,10]. The cause of Achilles
18
19 tendon rupture is unknown, but is thought to be based on mechanical
20
21 and biological factors [11].
22
23

24
25 Gray-scale US is routinely performed to evaluate tendinopathic
26
27 Achilles tendon, and has been used to assess the appearance of the
28
29 Achilles tendon following surgical repair [12-14]. Intratendinous
30
31 Doppler activity has been interpreted as an equivalent of neovessels
32
33 [15]. Colour- and power-Doppler highlight the increase of intra- and
34
35 peritendinous vascularisation in chronic tendon pathology [16]. Other
36
37 studies have shown similar results, and conclude that color/power
38
39 Doppler US is optimal to visualize soft-tissue hyperemia as a sign of
40
41 tendinopathy [17,18].
42
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45
46 Morphological studies showed increased microvascularisation and
47
48 failed healing response processes associated with Achilles tendon
49
50 rupture [19-21]. Zanetti et al. [3] observed a correlation between pain
51
52 and neovascularisation at power Doppler, but they did not demonstrate
53
54 an increased tendon rupture risk in symptomatic patients. Richards et
55
56 al. demonstrated with power-Doppler analysis a correlation between
57
58 increase of Achilles tendon vascularisation and chronic pain [22], and
59
60

1
2
3 showed that power Doppler is more sensitive than colour-Doppler to
4
5 study vascularity in tendon pathologies.
6

7
8 The presence of Doppler signal in the Achilles tendon does not
9
10 necessarily indicate disease [15]. At rest, healthy tendon do not
11
12 demonstrated increased blood flow at colour and power Doppler US.
13
14 Experimental studies with laser Doppler flowmetry (LDF) have
15
16 showed a minimum flux in healthy tendons [23]. Although the amount
17
18 of Doppler activity in non-symptomatic, healthy subjects was minute
19
20 compared with that of patient with chronic tendinopathy, a threshold
21
22 would be useful to distinguish normal from pathological
23
24 intratendinous Doppler activity. This threshold is machine-dependent
25
26 [20]. The vascular response to exercise in normal tendons evaluated
27
28 with colour Doppler has not been studied in detail [15]. However,
29
30 although colour Doppler flow in tendons is considered by some as a
31
32 sign of abnormality [13,17], colour Doppler flow is part of the
33
34 physiological response to exercise in Achilles tendons of healthy
35
36 normal subjects [15]. The tendon should not be evaluated immediately
37
38 after strenuous activity or eccentric exercise, because such an
39
40 increased flow could be present and produce a false positive reading
41
42 [15]. For this reason, we asked our subjects not to perform any sports
43
44 activity for at least two days before the US scan. However, one issue
45
46 is to be able to distinguish between physiological CD flow and
47
48 possible pathological flow, and cut off values must be defined.
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3 results of CEUS of the Achilles tendon in asymptomatic patients [24].
4

5 This approach allowed to detect in 5 healthy tendons the presence of
6
7 vessels in the central portion.
8

9
10 In our study, CEUS was more sensitive than power Doppler in
11
12 demonstrating tendon vascularisation. Indeed, we did not observe in
13
14 either group of subjects any Achilles tendon vascularisation with
15
16 power Doppler, while this became evident at CEUS from the
17
18 echogenicity of the micro bubbles of the contrast agents in the small
19
20 vessels, and Group A showed significantly higher vascularisation than
21
22 Group B. This suggested that, in high risk subjects for tendon
23
24 abnormalities with no clinical signs of tendinopathy and no US
25
26 changes, vascularisation is higher, although still within physiologic
27
28 limits (where physiologic should be considered no detectable signal at
29
30 power Doppler).
31
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35
36 The design of our study and the size of our cohorts do not allow to
37
38 speculate whether a rupture of the contralateral Achilles tendon will
39
40 occur in the subjects in group A, and whether the subjects of group B
41
42 will develop tendon pathology. Several variables can influence these
43
44 events, including the type and level of sports activity practiced.
45
46
47

48 Limits of our study include the lack of a reference method to verify
49
50 microvascularisation such as histological analysis or a LDF. In fact,
51
52 macroscopic and/or standard ultrasound evaluation might be unable to
53
54 detect tiny tendon changes visible only at histology [25]. Many factors
55
56 could have influenced our results, including cardiac output, the
57
58 technique of injection of the contrast medium, type and level of sport
59
60 activity practised. However, given the significant differences between

1
2
3 the two groups at CEUS, we suspect that a tendon vascularisation
4
5 threshold value could be predictive of a tissue degeneration. If you
6
7 could establish this value by using a reliable method, we would be
8
9 able to provide useful information, above all in professional athletes,
10
11 in order to identify initial tendinopathy in asymptomatic athletes with
12
13 no pathological changes observed at standard US. CEUS is at present
14
15 the only minimally-invasive method that allows to perform, through
16
17 enhancement curves, an accurate quantitative analysis of
18
19 vascularisation of the Achilles tendon. It is relatively easy from a
20
21 technical point of view, and is a quick and highly sensible method to
22
23 visualise tendon microcirculation in both healthy and symptomatic
24
25 subjects. There are no reported complications related to CEUS. . A
26
27 real clinical significance is still controversial, however at the moment
28
29 it could be reserved to professional athletes to identify a chronic
30
31 tendinopathy clinically silent. Further studies are needed to verify the
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33 prognostic implications of the increase of microvascularisation
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35 detected by CEUS to manage Achilles tendinopathy.
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46 REFERENCES

- 47
48 1. Reiter M, Ulreich N, Dirisamer A et al. Colour and power Doppler
49
50 sonography in symptomatic Achilles tendon disease. *Int J Sports Med*
51
52 2004;25(4):301-5.
53
54
55 2. Kristoffersen M, Ohberg L, Johnston C et al. Neovascularisation in
56
57 chronic tendon injuries detected with colour Doppler ultrasound in
58
59 horse and man: implications for research and treatment. *Knee Surg*
60
Sports Traumatol Arthrosc 2005;13(6):505-8.

- 1
2
3 3. Zanetti M, Metzdorf A, Kundert HP, et al. Achilles tendons: clinical
4
5
6 relevance of neovascularization diagnosed with power Doppler US.
7
8 *Radiology* 2003;227(2):556-60.
9
- 10 4. Albrecht T, Blomley M, Bolondi L, et al. Guidelines for the use of
11
12 contrast agents in ultrasound. January 2004. *Ultraschall Med*
13
14 2004;25(4):249-56.
15
16
- 17 5. Genovese EA, Callegari L, Combi F, et al. Contrast enhanced
18
19 ultrasound with second generation contrast agent for the follow-up of
20
21 lower-extremity muscle-strain-repairing processes in professional
22
23 athletes. *Radiol Med (Torino)* 2007;112(5):740-50.
24
25
- 26 6. Harvey CJ, Blomley MJ, Eckersley RJ et al. Developments in
27
28 ultrasound contrast media. *Eur Radiol* 2001;11(4):675-89.
29
30
- 31 7. Thorelius L. Contrast-enhanced ultrasound: beyond the liver. *Eur*
32
33 *Radiol* 2003;13 Suppl 3:N91-108.
34
35
- 36 8. Rudzki JR, Adler RS, Warren RF, et al. Contrast-enhanced
37
38 ultrasound characterization of the vascularity of the rotator cuff
39
40 tendon: age- and activity-related changes in the intact asymptomatic
41
42 rotator cuff. *J Shoulder Elbow Surg* 2008;17(1 Suppl):96S-100S.
43
44
- 45 9. Aroen A, Helgo D, Granlund OG et al. Contralateral tendon rupture
46
47 risk is increased in individuals with a previous Achilles tendon
48
49 rupture. *Scand J Med Sci Sports* 2004;14(1):30-3.
50
51
- 52 10. Maffulli N, Kenward MG, Testa V et al. Clinical diagnosis of
53
54 Achilles tendinopathy with tendinosis. *Clin J Sport Med*
55
56 2003;13(1):11-5.
57
58
59
60

- 1
2
3 11. Khan KM, Cook JL, Bonar F et al. Histopathology of common
4
5 tendinopathies. Update and implications for clinical management.
6
7
8 *Sports Med* 1999;27(6):393-408.
9
10
11 12. Fornage BD. Achilles tendon: US examination. *Radiology*
12
13 1986;159(3):759-64.
14
15 13. Martinoli C, Derchi LE, Pastorino C et al. Analysis of echotexture
16
17 of tendons with US. *Radiology* 1993;186(3):839-43.
18
19
20 14. Mathieson JR, Connell DG, Cooperberg PL et al. Sonography of
21
22 the Achilles tendon and adjacent bursae. *AJR Am J Roentgenol*
23
24 1988;151(1):127-31.
25
26
27 15. Boesen MI, Koenig MJ, Torp-Pedersen S et al. Tendinopathy and
28
29 Doppler activity: the vascular response of the Achilles tendon to
30
31 exercise. *Scand J Med Sci Sports* 2006;16(6):463-9.
32
33
34 16. Richards PJ, Dheer AK, McCall IM. Achilles tendon (TA) size
35
36 and power Doppler ultrasound (PD) changes compared to MRI: a
37
38 preliminary observational study. *Clin Radiol* 2001;56(10):843-50.
39
40
41 17. Ohberg L, Lorentzon R, Alfredson H. Neovascularisation in
42
43 Achilles tendons with painful tendinosis but not in normal tendons: an
44
45 ultrasonographic investigation. *Knee Surg Sports Traumatol Arthrosc*
46
47 2001;9(4):233-8.
48
49
50 18. Terslev L, Qvistgaard E, Torp-Pedersen S et al. Ultrasound and
51
52 Power Doppler findings in jumper's knee – preliminary observations.
53
54 *Eur J Ultrasound* 2001;13(3):183-9.
55
56
57 19. Maffulli N, Barrass V, Ewen SW. Light microscopic histology of
58
59 achilles tendon ruptures. A comparison with unruptured tendons. *Am J*
60
Sports Med 2000;28(6):857-63.

- 1
2
3 20. Movin T, Gad A, Reinholt FP et al. Tendon pathology in long-
4 standing achillodynia. Biopsy findings in 40 patients. *Acta Orthop*
5
6
7
8 *Scand* 1997;68(2):170-5.
9
- 10 21. Tallon C, Maffulli N, Ewen SW. Ruptured Achilles tendons are
11 significantly more degenerated than tendinopathic tendons. *Med Sci*
12
13 *Sports Exerc* 2001;33(12):1983-90.
14
15
- 16 22. Richards PJ, Win T, Jones PW. The distribution of microvascular
17 response in Achilles tendonopathy assessed by colour and power
18
19 Doppler. *Skeletal Radiol* 2005;34(6):336-42.
20
21
22
- 23 23. Astrom M, Westlin N. Blood flow in the human Achilles tendon
24 assessed by laser Doppler flowmetry. *J Orthop Res* 1994;12(2):246-
25
26
27
28
29 52.
30
- 31 24. Koenig MJ, Torp-Pedersen S, Holmich P, et al. Ultrasound
32
33 Doppler of the Achilles tendon before and after injection of an
34
35 ultrasound contrast agent--findings in asymptomatic subjects.
36
37
38 *Ultraschall Med* 2007;28(1):52-6.
39
40
- 41 25. Alfredson H, Lorentzon M, Backman S et al. cDNA-arrays and
42
43 real-time quantitative PCR techniques in the investigation of chronic
44
45 Achilles tendinosis. *J Orthop Res* 2003;21(6):970-5.
46
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Table I: CEUS results of Group A calculated from enhancement curves

N, gender, age (yrs)	[Peak]	[TTP]	[RBV]	[RBF]
1. M 27	4.9	47.9	379.7	5.1
2. M 28	4.4	44.7	588.7	4.8
3. M 29	Not assessable			
4. F 29	6.8	40.9	393.9	6.5
5. M 30	2.9	31.9	131.6	2.9
6. M 30	5.00	39.6	288.2	5.1
7. M 31	Not assessable			
8. F 32	3.9	37.7	254.7	4.2
9. M 32	3.5	40.7	191.6	3.5
10. M 33	2.9	46.5	208.8	3.0
11. M 34	6.0	38.1	346.1	6.2
12. M 36	3.4	40.2	212.1	3.5
13. M 37	1.9	38.8	133.9	2.1
14. M 47	1.6	30.7	76.8	1.9

PEAK: maximum signal intensity value;

TTP (Time to Peak): time that the contrast agent takes to reach its higher concentration (seconds);

RBV (Regional Blood Volume): blood volume proportional to the area under the curve (AUC), calculated as the integral of the same curve (ml);

RBF (Regional Blood Flow): blood flow, calculated as the ratio between RBV and MTT (medium transit time) (ml/seconds)

Table II: CEUS results of Group B calculated from enhancement curves

N, gender, age (yrs)	[Peak]	[TTP]	[RBV]	[RBF]
1. M 27	4.0	36.1	223.4	4.30
2. F 28	4.1	20.2	155.4	4.6
3. F 29	4.1	28.3	229.7	4.4
4. M 30	2.8	40.1	197.6	2.9
5. M 31	2.9	31.9	131.6	2.9
6. F 34	1.5	29.8	72.6	1.8
7. M 35	1.6	31.0	75.7	1.7
8. M 36	1.9	33.8	125.4	2
9. M 37	3.1	39.7	183.6	3.0
10. M 40	2.5	28.5	111.6	2.5

PEAK: maximum signal intensity value;

TTP (Time to Peak): time that the contrast agent takes to reach its higher concentration;

RBV (Regional Blood Volume): blood volume proportional to the area under the curve (AUC), calculated as the integral of the same curve;

RBF (Regional Blood Flow): blood flow, calculated as the ratio between RBV and MTT (medium transit time).

Analysis of Achilles tendon

microvasculature vascularity with second generation Contrast-Enhanced Ultrasound (CEUS)

ABSTRACT

Purpose: To compare morphological and power Doppler features of the Achilles tendon and Contrast-Enhanced Ultrasound (CEUS) behaviour between asymptomatic athletes and athletes who had undergone surgery for repair of a previous Achilles tendon rupture.

Methods: 24 athletes were divided in two groups (A and B). Group A included 14 patients with a median age of 32 years (range 27 to 47 years) who had undergone surgical repair for unilateral Achilles tendon rupture. Group B (control group) included 10 subjects with a median of 34 years (range 27 to 40 years) with no previous or present history of tendinopathy. All patients were evaluated with ultrasound (US), power-Doppler and CEUS with second-generation contrast agent. We studied the uninjured Achilles tendon in athletes who had suffered a previous rupture (Group A) and either the left or right Achilles tendon of the athletes in Group B. [Time-Intensity enhancement curves were calculated.](#)

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2
3 **Results:** CEUS showed a significantly greater ability to detect a
4 greater number of vascular spots within the tendon of Group A
5 compared to Group B (< 0.05).
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10 **Conclusions:** In athletes who had suffered a tear of an Achilles
11 tendon, CEUS detected small vessels that were not identified by
12 power-Doppler US in the uninjured controlateral Achilles tendon.
13 CEUS is useful to evaluate [microvascularisation](#) [vascularity](#) not
14 detected by other imaging techniques. [Vascularity seems to be](#)
15 [increased in patients who had suffered from a previous rupture.](#)
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26 INTRODUCTION

27 ~~B-mode Ultrasound (US) with colour and power Doppler is~~
28 ~~considered the only investigation able to evaluate~~
29 ~~microvascularisation.~~
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35 Many authors described a direct relationship between tendinopathy
36 and increased vascularisation in Achilles tendons, even though this
37 relationship is controversial [1-3]. Contrast-Enhanced Ultrasound
38 (CEUS) with second-generation contrast agent demonstrated a high
39 sensitivity to evaluate vascularity of different tissues [4-13 4-7].
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48 Recently, Rudzki and co-authors have used a contrast-enhanced
49 ultrasound method to demonstrate regional variations in the
50 vascularity of the supraspinatus tendon, with an age-dependent
51 decrease in asymptomatic individuals with intact rotator cuffs [14 8].
52
53 Patients who suffer from an acute Achilles tendon rupture have a
54 nearly 200-fold increased risk of a contralateral tendon rupture [15 9]
55 and many exhibit intratendinous changes in the contralateral Achilles
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3 tendon. This observational study compared the sensitivity of power
4
5 Doppler US and CEUS to assess the vascularisation of the Achilles
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7 tendon in two different populations. ~~The first one was composed by~~
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9 ~~subject who had sustained an Achilles tendon rupture and the~~
10
11 ~~contralateral tendon was assessed, and the second one was a healthy~~
12
13 ~~control group the tendon of the same side has been studied. We~~
14
15 ~~hypothesised that CEUS has a higher sensitivity than power Doppler~~
16
17 ~~US.~~

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21
22 The first one was composed by subject who had sustained an Achilles
23
24 tendon rupture and in which the contralateral tendon was assessed,
25
26 and the second one was a healthy control group in which either the
27
28 right or left healthy tendon was studied. We wanted to verify whether
29
30 CEUS has a higher sensitivity than power Doppler US in detecting
31
32 vasculature and that vascularity is increased in patients who suffered
33
34 from a previous rupture.

40 41 MATERIALS AND METHODS

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43 All the procedures described in the present study were approved by
44
45 our Institutional Review Board, and all participants gave written
46
47 informed consent to participate in the study.

48
49 Twenty-four amateur runners were included in the study; all athletes
50
51 were running at least three times in the week for at least 15 km. They
52
53 were divided in two groups. Group A was composed of 14 patients, 12
54
55 males and 2 females, with a median age of 32 years (range 27 to 47
56
57 years). All had undergone surgery for a unilateral Achilles tendon
58
59 rupture in the past two years from the present study. None of them had
60

1
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3 symptoms from the controlateral tendon. Group B was composed of
4
5 10 asymptomatic subjects (7 males and 3 females, with median age of
6
7 34 years (range 27 to 40 years) with no previous tendon pathology.
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9
10 The non affected Achilles tendon of group A subjects, and either the
11
12 left or the right Achilles tendon (selected by the subject tossing a coin
13
14 immediately before undergoing the ultrasound examination) of group
15
16 B subjects were studied with ~~B-mode~~ gray-scale US, power Doppler
17
18 and CEUS.
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20 All subjects were asked to abstain from sports activities for two days
21
22 before the evaluation. The exams were conducted with a Technos
23
24 MPX US (Esaote, Genova, Italy) by the senior radiologist with the
25
26 patient prone and the ankle at 90°; he was blinded to knowing whether
27
28 a patient belonged to group A or B. A gray-scale US and power-
29
30 Doppler with a ~~13-14~~ 13-4 MHz linear probe, with longitudinal and
31
32 transversal ~~scansions~~ scans, were used to detect any structural tendon
33
34 abnormalities. Anteroposterior thickness, US-~~structure~~ echotexture
35
36 (homogeneous / dishomogeneous), and signs of peritendinopathy were
37
38 evaluated at the middle one third and insertion of the Achilles tendon
39
40 on the calcaneus. Patients who showed clinical signs of tendinopathy
41
42 were excluded from the study. ~~For power Doppler evaluation, the gain~~
43
44 ~~was set to cancel any deep flow signals at the level of the calcaneal~~
45
46 ~~tuberosity.~~ For power Doppler evaluation, the gain was set to cancel
47
48 any noise signal. CEUS was then performed using longitudinal scans,
49
50
51 with a linear ~~9-13~~ 9-3 MHz probe and dedicated CnTi software
52
53 (Esaote, Genova, Italy). The radiologist administered a 5 ml volume
54
55 injection of second-generation contrast agent (Sonovue, Bracco,
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1
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3 Milano, Italy) followed by 10 ml saline solution in an antecubital vein
4
5 of the forearm of all the subjects involved in the present study. Two
6
7 videoclips of 60 seconds each were recorded, beginning at the end of
8
9 the injection of the contrast agent. The level of contrast diffusion in
10
11 the tendon was evaluated in a quantitative fashion drawing ~~intensity-~~
12
13 ~~time-~~ time intensity enhancement curves, using Qontrast™ software
14
15 (Bracco, Milano, Italy). The intensity of contrast diffusion in the
16
17 tendon was calculated on longitudinal tendon scan. The variables
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19 analyzed on the curves were the following:
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24 PEAK: maximum signal intensity value;

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26 TTP (Time to Peak): time that the contrast agent takes to reach its
27
28 higher concentration;

29
30 RBV (Regional Blood Volume): blood volume proportional to the
31
32 area under the curve

33
34 (AUC), calculated as the integral of the same curve;

35
36 RBF (Regional Blood Flow): blood flow, calculated as the ratio
37
38 between RBV and MTT (medium transit time).

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40 Data were entered in a commercially available database software.

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42 Descriptive statistics were calculated. All analyses were performed
43
44 using the SPSS 13.0 statistical software package (SPSS Science Inc.,
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46 Chicago, IL). A Mann-Whitney's test for independent samples was
47
48 used, considering significative a p value < 0.05.
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RESULTS

The gray-scale analysis did not show any pathological thickening at the middle third and the region of insertion on the calcaneus site of all the tendons evaluated. The anterior-posterior mean diameter was 4.6 ± 3.10 mm in group A and 4.55 ± 3.02 mm in the group B. The tendon structure was homogeneous without any focal hypo- or hyper-echoic lesions. We did not detect oedema in the tendon. Power Doppler exam did not show any intra-tendinous neovascularisation in either group. The results of CEUS are summarized in Tables I and II. The enhancement curves were drawn considering the data of the second videoclip because the presence of the contrast agent was appreciable in all subjects only at 40-60 seconds from the injection. In two male patients of group A, it was not possible to calculate the enhancement curve due to movement artefacts. Group A subjects showed a values of peak (mean 3,9; SD 0,07), TTP (mean 40,2; SD 5,1), RBV (mean 267; SD 142) significant higher than Group B subjects (peak mean 2,8; SD 1,07; TTP mean 31,9; SD 5,9; RBV mean 150; SD 56,8) with a $p < 0.0$. No difference was observed for the RBF parameter (mean 4,1; SD 1,5 in Group A and mean 3 with SD of 1,1 in Group B).

DISCUSSION

Midsubstance ruptures of the Achilles tendon typically occur in 30–50 year old male recreational athletes [15,17 9,10]. The cause of Achilles tendon rupture is unknown, but is thought to be based on mechanical and biological factors [18,19 11].

1
2
3 However, the aetiology and pathogenesis of failed healing response
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5 evident at histological examination of specimens from these patients
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7 remains to be clarified [20]. Overuse and repetitive loading appear to
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9 play a significant role [21]. The classical failed healing response at the
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11 basis of tendinopathy lesions, however, is also seen in inactive
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13 individuals [22].
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17 Gray-scale US is routinely performed to evaluate tendinopathic
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19 Achilles tendon, and has been used to assess the appearance of the
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21 Achilles tendon following surgical repair [23-28 12-14].
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25 Intratendinous Doppler activity has been interpreted as an equivalent
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27 of neovessels [20 15]. Colour- and power-Doppler highlight the
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29 increase of intra- and peritendinous vascularisation in chronic tendon
30
31 pathology [1,29 16]. Other studies have shown similar results, and
32
33 conclude that color/power Doppler US is optimal to visualize soft-
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35 tissue hyperemia as a sign of tendinopathy [20,30,31 17,18] and that it
36
37 could be used to quantify disease activity during treatment[20].
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41 Morphological studies showed increased microvascularisation and
42
43 failed healing response processes associated with Achilles tendon
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45 rupture [32-34 19-21]. Zanetti et al. [3] observed a correlation
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47 between pain and neovascularisation at power Doppler, but they did
48
49 not demonstrate an increased tendon rupture risk in symptomatic
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51 patients. Richards et al. demonstrated with power-Doppler analysis a
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53 correlation between increase of Achilles tendon vascularisation and
54
55 chronic pain [35 22], and showed that power Doppler is more
56
57 sensitive than colour-Doppler to study vascularity in tendon
58
59 pathologies.
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1
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3 ~~The neovascularisation appeared to be more likely related to the size~~
4 ~~of the tendon rather than the symptoms. They speculated that the~~
5 ~~progression to tendinopathy begins with the increase in tendon size~~
6 ~~followed by detection of blood flow at power Doppler evaluation, and~~
7 ~~then by pain [29]. Whether pain in Achilles tendon and intratendinous~~
8 ~~Doppler flow are predisposing signs of partial or complete rupture, or~~
9 ~~part of a healing phase is not known [18].~~

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20 The presence of Doppler signal in the Achilles tendon does not
21 necessarily indicate disease [20 15]. At rest, healthy tendon do not
22 demonstrated increased blood flow at colour and power Doppler US.
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27 Experimental studies with laser Doppler flowmetry (LDF) have
28 showed a minimum flux in healthy tendons [36, 37 23]. Although the
29 amount of Doppler activity in non-symptomatic, healthy subjects was
30 tiny compared with that of patient with chronic tendinopathy, a
31 threshold would be useful to distinguish normal from pathological
32 intratendinous Doppler activity. This threshold is machine-dependent
33 [20]. The vascular response to exercise in normal tendons evaluated
34 with colour Doppler has not been studied in detail [18 15]. However,
35 although colour Doppler flow in tendons is considered by some as a
36 sign of abnormality [27, 30 13,17], colour Doppler flow is part of the
37 physiological response to exercise in Achilles tendons of healthy
38 normal subjects [48, 20 15]. The tendon should not be evaluated
39 immediately after strenuous activity or eccentric exercise, because
40 such an increased flow could be present and produce a false positive
41 reading [20 15]. For this reason, we asked our subjects not to perform
42 any sports activity for at least two days before the US scan. However,

1
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3 one issue is to be able to distinguish between physiological CD flow
4 and possible pathological flow, and cut off values must be defined.
5

6
7 CEUS is a new procedure that allows to evaluate intra- and peri-
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9 tendinous microcirculation, being able to show vessels up to 40 μm in
10
11 diameter. To the best of our knowledge, only one study details the
12
13 results of CEUS of the Achilles tendon in asymptomatic patients [38
14
15 [24](#)]. This approach allowed to detect in 5 healthy tendons the presence
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17 of vessels in the central portion, ~~where the velocimetric spectrum did~~
18
19 ~~not include a diastolic component a resistance index equal to 1.00.~~
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23
24 In our study, CEUS was more sensitive than power Doppler in
25
26 demonstrating tendon vascularisation. Indeed, we did not observe in
27
28 either group of subjects any Achilles tendon vascularisation with
29
30 power Doppler, while this became evident at CEUS from the
31
32 echogenicity of the micro bubbles of the contrast agents in the small
33
34 vessels, and Group A showed significantly higher vascularisation than
35
36 Group B. This suggested that, in high risk subjects for tendon
37
38 abnormalities with no clinical signs of tendinopathy and no US
39
40 changes, vascularisation is higher, [although still within physiologic](#)
41
42 [limits \(where physiologic should be considered no detectable signal at](#)
43
44 [power Doppler\). Increased vascularisation can be considered as a risk](#)
45
46 [factor of tendinopathy \[2\], or can be a normal physiological response](#)
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48 [to physical activity\[39\].](#)
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55 The design of our study and the size of our cohorts do not allow to
56
57 speculate whether a rupture of the contralateral Achilles tendon will
58
59 occur in the subjects in group A, and whether the subjects of group B
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1
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3 will develop tendon pathology. Several variables can influence these
4
5 events, including the type and level of sports activity practiced.
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7
8 Limits of our study include the lack of a reference method to verify
9
10 microvascularisation such as histological analysis or a LDF. In fact,
11
12 macroscopic and/or standard ultrasound evaluation might be unable to
13
14 detect tiny tendon changes visible only at histology [40,41 25]. Many
15
16 factors could have influenced our results, including cardiac output, the
17
18 technique of injection of the contrast medium, type and level of sport
19
20 activity practised. However, given the significant differences between
21
22 the two groups at CEUS, ~~we suspect that a tendon vascularisation~~
23
24 ~~limit value could be predictive of a tissue lesion. A reliable method to~~
25
26 ~~detect this value can be useful to identify initial tendinopathy in~~
27
28 ~~asymptomatic athletes with no pathological changes observed at~~
29
30 ~~standard US.~~ we suspect that a tendon vascularisation threshold value
31
32 could be predictive of a tissue degeneration. If you could establish
33
34 this value by using a reliable method, we would be able to provide
35
36 useful information, above all in professional athletes, in order to
37
38 identify initial tendinopathy in asymptomatic athletes with no
39
40 pathological changes observed at standard US. CEUS is at present the
41
42 only ~~non~~ minimally-invasive method that allows to perform, through
43
44 enhancement curves, an accurate quantitative analysis of
45
46 vascularisation of the Achilles tendon. It is relatively easy from a
47
48 technical point of view, and is a quick and highly sensible method to
49
50 visualise tendon microcirculation in both healthy and symptomatic
51
52 subjects. There are no reported complications related to CEUS. A real
53
54 clinical significance is still controversial, however at the moment it
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3 [could be reserved to professional athletes to identify a chronic](#)
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5 [tendinopathy clinically silent.](#) Further studies are needed to verify the
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8 prognostic implications of the increase of microvascularisation
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10 detected by CEUS to manage Achilles tendinopathy.
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14 REFERENCES

- 15
16
17 1. Reiter M, Ulreich N, Dirisamer A et al. Colour and power Doppler
18 sonography in symptomatic Achilles tendon disease. *Int J Sports Med*
19 2004;25(4):301-5.
20
21
22 2. Kristoffersen M, Ohberg L, Johnston C et al. Neovascularisation in
23 chronic tendon injuries detected with colour Doppler ultrasound in
24 horse and man: implications for research and treatment. *Knee Surg*
25 *Sports Traumatol Arthrosc* 2005;13(6):505-8.
26
27
28 3. Zanetti M, Metzdorf A, Kundert HP, et al. Achilles tendons: clinical
29 relevance of neovascularization diagnosed with power Doppler US.
30 *Radiology* 2003;227(2):556-60.
31
32
33 4. Agati L, De Maio F, Madonna MP et al. Tailored reperfusion
34 strategies in acute myocardial infarction : role of intravenous
35 myocardial contrast echocardiography. *Echocardiography* 2002;
36 19(7):627-34.
37
38
39 5. Albrecht T, Blomley M, Bolondi L, et al. Guidelines for the use of
40 contrast agents in ultrasound. January 2004. *Ultraschall Med*
41 2004;25(4):249-56.
42
43
44 6. Burns PN, Wilson SR, Simpson DH. Pulse inversion imaging of
45 liver blood flow: improved method for characterizing focal masses
46 with microbubble contrast. *Invest Radiol* 2000; 35(1):58-71.
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 7. Carrafiello G, Laganà D, Recaldini C, et al. Comparison of
4
5
6 contrast-enhanced ultrasound and computed tomography in classifying
7
8
9 endoleaks after endovascular treatment of abdominal aorta aneurysms:
10
11 preliminary experience. *Cardiovasc Intervent Radiol* 2006; 29(6):969-
12
13 74.
- 14
15 8. Catalano O, Iobianco R, Raso MM et al. Blunt hepatic trauma:
16
17
18 evaluation with contrast-enhanced sonography: sonographic findings
19
20 and clinical application. *J Ultrasound Med* 2005; 24(3):299-310.
- 21
22 9. Genovese EA, Callegari L, Combi F, et al. Contrast-enhanced
23
24
25 ultrasound with second-generation contrast agent for the follow-up of
26
27
28 lower-extremity muscle-strain-repairing processes in professional
29
30 athletes. *Radiol Med (Torino)* 2007; 112(5):740-50.
- 31
32 10. Harvey CJ, Blomley MJ, Eckersley RJ et al. Developments in
33
34
35 ultrasound contrast media. *Eur Radiol* 2001; 11(4):675-89.
- 36
37 11. Hohmann J, Skrok J, Puls R et al. Characterization of focal liver
38
39
40 lesions with contrast-enhanced low-MI real-time ultrasound and
41
42 SonoVue. *Rofo* 2003; 175(6):835-43.
- 43
44 12. Lencioni R. Impact of European Federation of Societies for
45
46
47 Ultrasound in Medicine and Biology (EFSUMB) guidelines on the use
48
49
50 of contrast agents in liver ultrasound. *Eur Radiol* 2006; 16(7):1610-3.
- 51
52 13. Thorelius L. Contrast-enhanced ultrasound: beyond the liver. *Eur*
53
54
55 *Radiol* 2003; 13 Suppl 3:N91-108.
- 56
57 14. Rudzki JR, Adler RS, Warren RF, et al. Contrast-enhanced
58
59
60 ultrasound characterization of the vascularity of the rotator cuff
tendon: age- and activity-related changes in the intact asymptomatic
rotator cuff. *J Shoulder Elbow Surg* 2008; 17(1 Suppl):96S-100S.

- 1
2
3 15. Aroen A, Helgo D, Granlund OG et al. Contralateral tendon
4 rupture risk is increased in individuals with a previous Achilles tendon
5
6 rupture. *Scand J Med Sci Sports* 2004;14(1):30-3.
7
8
9
10 16. Maffulli N, Kenward MG, Testa V et al. Clinical diagnosis of
11
12 Achilles tendinopathy with tendinosis. *Clin J Sport Med*
13
14 2003;13(1):11-5.
15
16
17 17. Maffulli N. rupture of the Achilles tendon. *J Bone Joint Surg Am*
18
19 1999;81(7):1019-36
20
21
22 18 Boesen MI, Boesen A, Koenig MJ et al. Ultrasonographic
23
24 investigation in the Achilles tendon in elite badminton players using
25
26 color Doppler. *Am J Sports Med* 2006;34(12):2013-21.
27
28
29 19. Khan KM, Cook JL, Bonar F et al. Histopathology of common
30
31 tendinopathies. Update and implications for clinical management.
32
33 *Sports Med* 1999;27(6):393-408.
34
35
36 20 Boesen MI, Koenig MJ, Torp-Pedersen S et al. Tendinopathy and
37
38 Doppler activity: the vascular response of the Achilles tendon to
39
40 exercise. *Scand J Med Sci Sports* 2006;16(6):463-9.
41
42
43 21. Archambault JM, Wiley JP, Bray RC. Exercise loading of tendons
44
45 and the development of overuse injuries. A review of current
46
47 literature. *Sports Med* 1995;20(2):77-89.
48
49
50 22 Alfredson H, Lorentzon R. Chronic Achilles tendinosis:
51
52 recommendations for treatment and prevention. *Sports Med*
53
54 2000;29(2):135-6.
55
56
57 23. Fornage BD. Achilles tendon: US examination. *Radiology*
58
59 1986;159(3):759-64.
60

- 1
2
3 24. Maffulli N, Dymond NP, Capasso G. Ultrasonographic findings in
4 subcutaneous rupture of Achilles tendon. *J Sports Med Phys Fitness*
5 1989;29(4):365-8.
6
7
8
9
10 25. Maffulli N, Dymond NP, Regine R. Surgical repair of ruptured
11 Achilles tendon in sportsmen and sedentary patients: a longitudinal
12 ultrasound assessment. *Int J Sports Med* 1990;11(1):78-84.
13
14
15
16
17 26. Maffulli N, Regine R, Angelillo M et al. Ultrasound diagnosis of
18 Achilles tendon pathology in runners. *Br J Sports Med*
19 1987;21(4):158-62.
20
21
22
23
24 27. Martinoli C, Derchi LE, Pastorino C et al. Analysis of echotexture
25 of tendons with US. *Radiology* 1993;186(3):839-43.
26
27
28
29 28. Mathieson JR, Connell DG, Cooperberg PL et al. Sonography of
30 the Achilles tendon and adjacent bursae. *AJR Am J Roentgenol*
31 1988;151(1):127-31.
32
33
34
35
36 29. Richards PJ, Dheer AK, McCall IM. Achilles tendon (TA) size
37 and power Doppler ultrasound (PD) changes compared to MRI: a
38 preliminary observational study. *Clin Radiol* 2001;56(10):843-50.
39
40
41
42
43 30. Ohberg L, Lorentzon R, Alfredson H. Neovascularisation in
44 Achilles tendons with painful tendinosis but not in normal tendons: an
45 ultrasonographic investigation. *Knee Surg Sports Traumatol Arthrosc*
46 2001;9(4):233-8.
47
48
49
50
51
52
53 31. Terslev L, Qvistgaard E, Torp-Pedersen S et al. Ultrasound and
54 Power Doppler findings in jumper's knee—preliminary observations.
55
56
57
58
59
60

- 1
2
3 32. Maffulli N, Barrass V, Ewen SW. Light microscopic histology of
4 achilles tendon ruptures. A comparison with unruptured tendons. *Am J*
5
6 *Sports Med* 2000;28(6):857-63.
7
8
9
10 33. Movin T, Gad A, Reinholt FP et al. Tendon pathology in long-
11 standing achillodynia. Biopsy findings in 40 patients. *Acta Orthop*
12
13 *Scand* 1997;68(2):170-5.
14
15
16
17 34. Tallon C, Maffulli N, Ewen SW. Ruptured Achilles tendons are
18 significantly more degenerated than tendinopathic tendons. *Med Sci*
19
20 *Sports Exerc* 2001;33(12):1983-90.
21
22
23
24 35. Richards PJ, Win T, Jones PW. The distribution of microvascular
25 response in Achilles tendonopathy assessed by colour and power
26
27 Doppler. *Skeletal Radiol* 2005;34(6):336-42.
28
29
30
31 36. Astrom M, Westlin N. Blood flow in chronic Achilles
32 tendonopathy. *Clin OrthopRelat Res* 1994(308):166-72.
33
34
35
36 37. Astrom M, Westlin N. Blood flow in the human Achilles tendon
37 assessed by laser Doppler flowmetry. *J Orthop Res* 1994;12(2):246-
38
39 52.
40
41
42
43 38. Koenig MJ, Torp-Pedersen S, Holmich P, et al. Ultrasound
44 Doppler of the Achilles tendon before and after injection of an
45
46 ultrasound contrast agent—findings in asymptomatic subjects.
47
48 *Ultraschall Med* 2007;28(1):52-6.
49
50
51
52
53 39. Malliaras P, Richards PJ, Garau G et al. Achilles tendon Doppler
54
55 flow may be associated with mechanical load among active athletes.
56
57 *Am J Sports Med* 2008;36:2210-2215.
58
59
60

1
2
3 40. Alfredson H, Lorentzon M, Backman S et al. cDNA arrays and
4
5 real time quantitative PCR techniques in the investigation of chronic
6
7 Achilles tendinosis. *J Orthop Res* 2003;21(6):970-5.

8
9
10 41. de Mos M, van El B, Degroot J et al. Achilles tendinosis: changes
11
12 in biochemical composition and collagen turnover rate. *Am J Sports*
13
14 *Med* 2007;35(9):1549-56.

15
16
17
18
19
20
21
22 1. Reiter M, Ulreich N, Dirisamer A et al. Colour and power Doppler
23
24 sonography in symptomatic Achilles tendon disease. *Int J Sports Med*
25
26 *2004;25(4):301-5.*

27
28
29 2. Kristoffersen M, Ohberg L, Johnston C et al. Neovascularisation in
30
31 chronic tendon injuries detected with colour Doppler ultrasound in
32
33 horse and man: implications for research and treatment. *Knee Surg*
34
35 *Sports Traumatol Arthrosc* 2005;13(6):505-8.

36
37
38 3. Zanetti M, Metzdorf A, Kundert HP, et al. Achilles tendons: clinical
39
40 relevance of neovascularization diagnosed with power Doppler US.
41
42 *Radiology* 2003;227(2):556-60.

43
44
45 4. Albrecht T, Blomley M, Bolondi L, et al. Guidelines for the use of
46
47 contrast agents in ultrasound. January 2004. *Ultraschall Med*
48
49 *2004;25(4):249-56.*

50
51
52 5. Genovese EA, Callegari L, Combi F, et al. Contrast enhanced
53
54 ultrasound with second generation contrast agent for the follow-up of
55
56 lower-extremity muscle-strain-repairing processes in professional
57
58 athletes. *Radiol Med (Torino)* 2007;112(5):740-50.

- 1
2
3 [6. Harvey CJ, Blomley MJ, Eckersley RJ et al. Developments in](#)
4 [ultrasound contrast media. *Eur Radiol* 2001;11\(4\):675-89.](#)
5
6
- 7 [7. Thorelius L. Contrast-enhanced ultrasound: beyond the liver. *Eur*](#)
8 [*Radiol* 2003;13 Suppl 3:N91-108.](#)
9
10
- 11 [8. Rudzki JR, Adler RS, Warren RF, et al. Contrast-enhanced](#)
12 [ultrasound characterization of the vascularity of the rotator cuff](#)
13 [tendon: age- and activity-related changes in the intact asymptomatic](#)
14 [rotator cuff. *J Shoulder Elbow Surg* 2008;17\(1 Suppl\):96S-100S.](#)
15
16
- 17 [9. Aroen A, Helgo D, Granlund OG et al. Contralateral tendon rupture](#)
18 [risk is increased in individuals with a previous Achilles tendon](#)
19 [rupture. *Scand J Med Sci Sports* 2004;14\(1\):30-3.](#)
20
21
- 22 [10. Maffulli N, Kenward MG, Testa V et al. Clinical diagnosis of](#)
23 [Achilles tendinopathy with tendinosis. *Clin J Sport Med*](#)
24 [2003;13\(1\):11-5.](#)
25
26
- 27 [11. Khan KM, Cook JL, Bonar F et al. Histopathology of common](#)
28 [tendinopathies. Update and implications for clinical management.](#)
29 [*Sports Med* 1999;27\(6\):393-408.](#)
30
31
- 32 [12. Fornage BD. Achilles tendon: US examination. *Radiology*](#)
33 [1986;159\(3\):759-64.](#)
34
35
- 36 [13. Martinoli C, Derchi LE, Pastorino C et al. Analysis of echotexture](#)
37 [of tendons with US. *Radiology* 1993;186\(3\):839-43.](#)
38
39
- 40 [14. Mathieson JR, Connell DG, Cooperberg PL et al. Sonography of](#)
41 [the Achilles tendon and adjacent bursae. *AJR Am J Roentgenol*](#)
42 [1988;151\(1\):127-31.](#)
43
44
45
46
47
48
49
50
51
52
53
54
55
56
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58
59
60

- 1
2
3 15. Boesen MI, Koenig MJ, Torp-Pedersen S et al. Tendinopathy and
4 Doppler activity: the vascular response of the Achilles tendon to
5 exercise. *Scand J Med Sci Sports* 2006;16(6):463-9.
6
7
8
9
10 16. Richards PJ, Dheer AK, McCall IM. Achilles tendon (TA) size
11 and power Doppler ultrasound (PD) changes compared to MRI: a
12 preliminary observational study. *Clin Radiol* 2001;56(10):843-50.
13
14
15
16
17 17. Ohberg L, Lorentzon R, Alfredson H. Neovascularisation in
18 Achilles tendons with painful tendinosis but not in normal tendons: an
19 ultrasonographic investigation. *Knee Surg Sports Traumatol Arthrosc*
20 2001;9(4):233-8.
21
22
23
24
25
26
27 18. Terslev L, Qvistgaard E, Torp-Pedersen S et al. Ultrasound and
28 Power Doppler findings in jumper's knee – preliminary observations.
29 *Eur J Ultrasound* 2001;13(3):183-9.
30
31
32
33
34 19. Maffulli N, Barrass V, Ewen SW. Light microscopic histology of
35 achilles tendon ruptures. A comparison with unruptured tendons. *Am J*
36 *Sports Med* 2000;28(6):857-63.
37
38
39
40
41 20. Movin T, Gad A, Reinholt FP et al. Tendon pathology in long-
42 standing achillodynia. Biopsy findings in 40 patients. *Acta Orthop*
43 *Scand* 1997;68(2):170-5.
44
45
46
47
48 21. Tallon C, Maffulli N, Ewen SW. Ruptured Achilles tendons are
49 significantly more degenerated than tendinopathic tendons. *Med Sci*
50 *Sports Exerc* 2001;33(12):1983-90.
51
52
53
54
55 22. Richards PJ, Win T, Jones PW. The distribution of microvascular
56 response in Achilles tendonopathy assessed by colour and power
57 Doppler. *Skeletal Radiol* 2005;34(6):336-42.
58
59
60

1
2
3 [23. Astrom M, Westlin N. Blood flow in the human Achilles tendon](#)
4 [assessed by laser Doppler flowmetry. *J Orthop Res* 1994;12\(2\):246-](#)
5 [52.](#)
6
7
8

9
10 [24. Koenig MJ, Torp-Pedersen S, Holmich P, et al. Ultrasound](#)
11 [Doppler of the Achilles tendon before and after injection of an](#)
12 [ultrasound contrast agent--findings in asymptomatic subjects.](#)
13 [*Ultraschall Med* 2007;28\(1\):52-6.](#)
14
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19 [25. Alfredson H, Lorentzon M, Backman S et al. cDNA-arrays and](#)
20 [real-time quantitative PCR techniques in the investigation of chronic](#)
21 [Achilles tendinosis. *J Orthop Res* 2003;21\(6\):970-5.](#)
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3 Reply to Rewiever 1
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- 6
7 1) Was the senior radiologist blinded to knowing whether a particular
8 patient was group A or group B? This should be indicated.
9

10 The senior radiologist was blinded whether a patient belonged to group A or B.
11

- 12
13 2) In group A patients, we should be told how many (if any) had symptoms
14 related to the contralateral Achilles tendon, if any. It would be interesting to
15 subdivide group A patients into two groups; those with symptoms in the contralateral tendon and
16 those who do not, and look for differences.
17

18 None of the patient of group A had symptoms related to the controlateral tendon.
19

- 20
21 3) On page 9, line 53, contrast-enhanced US is referred to as a 'non-
22 invasive method'. I don't consider the procedure non-invasive. Most would consider
23 an intravenous injection to be at least minimally invasive. This relates to
24 the next point as well.
25

26 At least a brief comment should be made as to the potential clinical
27 utility of this technique in the future should be made. This is alluded to at the
28 beginning of page 9 (increased ability to detect microvascularization in
29 asymptomatic patient), but should be more explicitly addressed given that
30 it an invasive technique and that it cannot be done without patient
31 preparation (no physical activity for 48 hours prior to scanning).
32

33
34 I agree that CEUS could be considered as a minimally invasive procedure.

35 Our results are still very preliminary and need to be conformed on larger series. A possible field of
36 application could be in professional athletes who are at high risk of injuries in order to detect
37 asymptomatic degenerative lesion. We tried to better explain this point in the text.
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43 Reply to Comments from the Editor
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45
46 Title

- 47 3) The title was changed as suggested.
48

49 Abstract

50 4-5) We compared the vasculature detected by power Doppler and CEUS in healthy subjects and
51 subjects at risk and we observed that subjects at risk had significant higher vasculature detected by
52 CEUS only.
53
54

55 Introduction

56 6-7) It was changed as asked.
57

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59 Materials and Methods

60 8, 9) Details required were added.

12) The correct frequency transducer was written (it was a typing mistake).

10, 11, 13, 14) The sonographic terminology was checked.

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15) the sentence was reworded

16) We recorded two clips because it was easier to elaborate data on the software.

17, 18) it was changed as suggested

Results

19-22) We wrote the results in the section and eliminate the tables.

23) NS was written for mistake.

25-26) We observed that in all patients contrast enhancement in the Achilles tendon was visible only after 40-60 seconds; so the first 40 seconds of the clip are useless to calculate the data.

Discussion

Discussion was rewritten.

Illustrations were eliminated.

For Peer Review