

The “Gastrocnemius–Achilles Tendon–Calcaneus Complex”: Different Responses after Percutaneous versus Vulpius Achilles Tendon Lengthening in New Zealand White Rabbits

Abstract

Background: This study aimed to describe the clinical, radiological, biomechanical, electromyographic, and histoenzymologic modifications in the “Gastrocnemius–Achilles Tendon–Calcaneus complex” caused by percutaneous Achilles tendon lengthening (PATL) versus Vulpius Achilles tendon lengthening (VATL) in New Zealand White (NZW) rabbits. **Materials and Methods:** Eight female NZW rabbits were used at 7 months of age. Two rabbits were euthanized before surgery for anatomical dissection, three underwent PATL (two bilateral and one unilateral), and the three others underwent VATL (two bilateral and one unilateral). Clinical examination, biomechanics, electromyography, standard radiographs and magnetic resonance imaging (MRI), and histology and histoenzymology were assessed after surgery. **Results:** At the end of the experiment, the subjects showed good clinical status but different functional outcomes of surgery: rabbits submitted to PATL developed permanent limp and lost their capacity to jump compared to rabbits submitted to VATL which remained able to ambulate and jump normally. Standard radiographs and MRI showed that PATL led to significantly greater increase in dorsal or anterior flexion of the tibiotarsal angle (TT angle) compared to VATL, whereas electromyographic and histoenzymologic observations of muscle unit showed little or no variation between the two groups of operated rabbits. **Conclusions:** Although PATL leads to greater improvement in dorsal or anterior flexion (TT angle) of the rabbit ankle compared to VATL, it has negative effects on functional outcome as it reduces the contractile capacity of the rabbit muscle unit, ultimately impairing the ability to ambulate and jump.

Keywords: Comparison, congenital clubfoot, New Zealand White rabbits, percutaneous Achilles tendon lengthening, Vulpius lengthening

Introduction

Congenital clubfoot can be treated with serial casting and percutaneous Achilles tendon lengthening (PATL), also known as the Ponseti method,¹⁻⁵ or with serial manipulations and Vulpius Achilles tendon lengthening (VATL), also known as the French functional physical therapy method.^{6,7} Both the methods achieve similar clinical outcome, reducing the need for extensive surgery.⁸⁻¹⁰

Both the Ponseti and the French functional physical therapy methods include an early surgical procedure aiming to correct the residual equinus. In particular, the PATL in the Ponseti method lengthens the tendon at the level of its distal portion whereas the VATL in the French functional physical therapy method leaves the tendon intact but lengthens the fascia of the gastrocnemius

muscle.^{11,12} A mini-open technique for Achilles tenotomy in infants with congenital clubfoot has recently been proposed.^{13,14}

Rats, dogs, cats, and rabbits have been extensively used as animal models to study injury, surgery, and repair in the Achilles tendon.¹⁵ In particular, rabbits have been used widely in this research context as they are easily available, and their muscle–tendon unit is suitably sized for both histologic and histoenzymologic assessments, radiology, magnetic resonance imaging (MRI), and biomechanical and electromyographic testing.¹⁶⁻¹⁸ Moreover, rabbits and humans share a similar “Gastrocnemius–Achilles tendon–Calcaneus complex” based on gross anatomy observation and MRI studies,^{16,19-21} and both species are plantigrade and pentadactyl in different postures^{22,23} [Table 1].

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This study aimed to describe the clinical, radiological, biomechanical, electromyographic, and histoenzymologic modifications in the “Gastrocnemius–Achilles tendon–Calcaneus complex” caused by PATL versus VATL in New Zealand White (NZW) rabbits.

Materials and Methods

Operative procedures and animal care were carried out in compliance with all national (Italian regulation D.L.vo 116/1992) and international (regulation 86/609/EC) legislation. Before starting the study, the protocol was first examined and approved by the Institutional Review Board of the Animal Facility at the Department of Life Sciences, University of Trieste, Italy, and subsequently transferred and approved by the Ministry of Health, Rome, Italy, in compliance with national and international regulations (D.L.vo 26/2014 and D.va 2010/63/UE). We worked to the ARRIVE guidelines for reporting animal research.²⁴

Animals

This study used eight “germ-free” female NZW rabbits (Harlan Laboratories, Leicester, UK) housed in a climate-controlled environment (21°C temperature, 40%–50% relative humidity, 10–15 air changes per hour, and 12 h/12 h light/dark cycle) in stainless-steel cages. Cages measured 0.7 m in length, 0.43 m in height, and 0.53 m in depth, and cage volume was 0.17 m³. Rabbits were fed a standard pellet diet (2030 Global Rabbit Diet 2030, Harlan Laboratories, Indianapolis, IN, USA) and water *ad libitum*. A tattoo on the left ear served to identify the rabbits.

Two rabbits were euthanized before surgery for anatomical dissection, to study the lower leg anatomy of *Oryctolagus cuniculus* [Table 1].

Surgery was performed in six 7-month-old rabbits: three rabbits underwent PATL and the other three were operated according to the VATL technique.^{2,3,15-17}

Anesthesia, postoperative treatment, and euthanasia

Anesthesia

All surgeries were performed under general anesthesia (GA) obtained by an intramuscular injection of xylazine 2.5 mg/kg (Virbaxil® 2%, Virbac Laboratories, Carros, France) and tiletamine-zolazepam 7.5 mg/kg (Zoletil® 100, Virbac Laboratories, Carros, France). Subsequent local and cutaneous anesthesia was obtained with a subcutaneous injection of 2% lidocaine hydrochloride (1 ml/animal).

Postoperative treatment

In the postoperative period, pain was relieved by subcutaneous administration of carprofen (Rimadyl®, Pfizer Animal Health, West Dundee, UK; 5 mg/kg twice daily for 5 days). In the week following surgery, rabbits were administered an intramuscular injection of enrofloxacin (Baytril® 5%, Bayer Animal Health, Kiel, Germany; 5 mg/kg twice daily) to prevent infection.

Euthanasia

Rabbits were anesthetized with an intramuscular injection of xylazine 5 mg/kg (Virbaxil® 2%; Virbac Laboratories, Carros, France) and tiletamine-zolazepam 15 mg/kg (Zoletil® 100, Virbac Laboratories, Carros, France), euthanized with an intravenous injection of embutramide-mebenzoniotetracaine 26 mg/kg (Tanax®, Intervet Italia Srl, Peschiera Borromeo, Italy), then, after death, jugulated.

Operative procedure

Surgery was performed in six 7-month-old rabbits. Three rabbits underwent PATL, with bilateral lengthening in two cases and unilateral lengthening in one case. Three rabbits were operated according to the VATL technique, again with bilateral lengthening in two cases and unilateral lengthening in one case.^{2,5}

The protocol for each rabbit involved (i) prepping and draping of the lower extremity, (ii) PATL at the level of the distal portion¹ [Figure 1] or section of the fascia of the gastrocnemius muscle (VATL) leaving the Achilles

Table 1: Similarities and differences in the “Gastrocnemius-Achilles Tendon-Calcaneus complex” between New Zealand White rabbits and humans

	NZW rabbits	Humans
Gastrocnemius muscle	Big, long Two distinct tendons (lateral and medial head) fuse at 90% of their course, close to calcaneal insertion	Short Two distinct tendons (lateral and medial head) converge more proximally at 25% of their course to form the Achilles tendon (triceps surae) course
	Flexor digitorum superficialis, medial and lateral gastrocnemius tendon are almost the same size and share the same fascia	Flexor digitorum superficialis runs outside the triceps surae
Soleus muscle	Negligible	Present
Posture	Plantigrade	Plantigrade
Foot	Pentadactyl	Pentadactyl

NZW=New Zealand White

tendon intact^{11,12} [Figure 2], (iii) spica cast immobilization, and (iv) cast removal 3–4 weeks after index surgery.

Clinical evaluation

The weight of each rabbit was recorded before surgery, immediately after cast removal, and every 7–10 days thereafter until the end of the experiment, i.e., 4 months after surgery. Overall, body weight was assessed 14 times to monitor postoperative recovery and to assess well-being of rabbits regularly.

During the postoperative period, rabbits were clinically assessed every day while in cast and every 4–6 days after cast removal. Clinical evaluation regularly assessed ambulation, jumping capabilities, and tibiotarsal angle (TT) of each subject.

Electromyography

Each rabbit underwent one electromyographic evaluation with an electro-myograph (MV TO S/N 532 device; B Neuro S. P. A., Florence, Italy) performed 2 weeks after cast removal in all rabbits. The electrodes were placed at the medial and lateral head of the gastrocnemius and evaluated muscle fibers depolarization following electrical stimulation. Right and left sides were assessed alternately. Each measurement was performed twice in order to evaluate the presence of possible myopathy and/or muscle weakness.

Radiographic evaluation: Standard radiographs and magnetic resonance imaging

Standard radiographs and MRI were performed 4 months after surgery.

Lateral radiographs of the leg and foot were performed under GA in all rabbits. To standardize measurements, an elastic bandage was placed between the foot and leg, enabling the TT angle to be measured with constant tension applied at the level of the “Gastrocnemius–Achilles tendon–Calcaneus complex”. TT angle was defined as the angle between the main axis of the tibia and the main axis of the calcaneus.

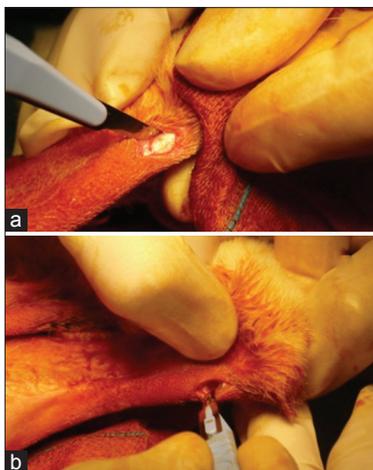


Figure 1: Small skin incision (a) and percutaneous Achilles tendon lengthening at the level of its distal portion (b)

All MRI scan examinations (T1 and T2 weighted) were performed on a 3T Bruker BioSpec MRI scanner (Bruker Biospin Corporation, Billerica, MA, USA) under GA with the lower extremity kept in neutral position. The MRI images served to perform an objective assessment of the healing of the “Gastrocnemius–Achilles tendon–Calcaneus complex.”

Histoenzymology and histology

Histoenzymology

Immediately after euthanasia–jugulation, 0.3–0.5 cm³, two to three tissue samples were harvested from the mid-portion of the gastrocnemius muscle belly of each lower extremity of all rabbits. Specimens were immediately mounted for transverse section on a cork disk frozen at –160°C in isopentane/liquid nitrogen cooling bath. Frozen blocks were conserved at –80°C and 3–5 μm sections were cut in a cryostat. Reactions for adenosine triphosphatase at pH 9.4 and preincubation at pH 4.35 and pH 4.63 were carried out to assess Type I, Type IIA, and Type IIB muscle fibers.

Histology

Immediately after euthanasia–jugulation, four 0.5–1 cm³ tissue samples (one per site), were harvested from the gastrocnemius muscle belly and Achilles tendon as follows: (a) transverse (muscle), (b) transverse (fascia), (c) longitudinal (Achilles tendon), and (d) transverse (Achilles tendon). Samples were fixed in 10% buffered formalin, embedded in paraffin, and microtomed to 8–10 μm-thick sections for routine hematein–eosin–safran staining.

Results

Body weight and clinical evaluation

Table 2 shows the weight changes in operated rabbits. Following surgery, all rabbits lost body weight. After cast removal, all rabbits gained body weight but without recovering their initial values before 4 months after index

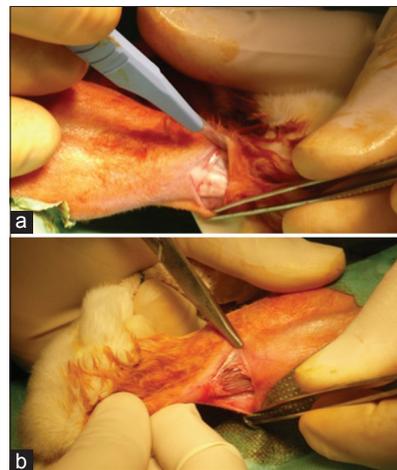


Figure 2: Exposure (a) and section (b) of the fascia of the Gastrocnemius muscle (Vulpus Achilles tendon lengthening), leaving the Achilles tendon intact

surgery [Table 2]. Weight variations were not significantly different between the two groups of rabbits ($P > 0.05$).

Table 3 outlines the clinical findings on all rabbits in postoperative followup [Table 3].

Electromyography

Electromyography assessment performed 2 weeks after cast removal found that all rabbits did not show any sign of myopathy or muscle weakness at the level of the lateral and medial head of the right and left gastrocnemius. Electromyography assessment was found to be within normal limits in all rabbits.

Radiographic evaluation

Table 4 shows the variations in TT angle of all operated rabbits [Table 4]. TT angle measured on standard lateral leg and foot radiographs of each subject [Figure 3] was significantly decreased in PATL compared to VATL rabbits ($P < 0.05$).

MRI was performed under GA on all rabbits at 4 months after index surgery with both legs in the same image to allow precise comparison between muscle-tendon units. MRI images showed complete healing of both Achilles tendon and gastrocnemius fascia [Figure 4]. MRI assessment found no signs of denervation in any of the rabbits.²¹

Histoenzymology and histology

Histoenzymology

Histoenzymology images gave a picture of the possible variations in distributions of the main muscle fiber

Types I, IIA, and IIB identified by myosin-ATPase activity: slow-twitch oxidative muscle fibers (Type I) and fast-twitch oxidative glycolytic (Type IIA and glycolytic Type IIB). Gross qualitative-only examination of histoenzymologic pictures did not reveal any difference between muscle fiber types of the nonoperated and operated rabbit legs (PATL vs. VATL) [Figure 5].

Histology

Histological observations on the muscle and tendon of both operated and nonoperated legs showed no structural difference in specimens from both groups of all rabbits that survived to reach the end of experiment: Muscle and tendon had normal microscopic anatomy and similar pictures in nonoperated legs and VATL animals only, whereas tendon rupture/resection remained in PATL animals [Figure 6].

Discussion

This experimental study aimed to compare the outcome of PATL and VATL in NZW rabbits. Animals were evaluated clinically, radiologically, and with electromyography, and evaluation was completed with histology and histoenzymology of muscle and tendon specimens. Although PATL and VATL had comparable histologic, histoenzymologic, and electromyographic results, they had different clinical and radiological outcome [Table 5].

Pre- and postoperative measurements of TT angle showed that PATL modifies TT angle more than VATL. TT angle in rabbits submitted to PATL varied by about 40° clinically and 30° radiologically, while TT angle in rabbits

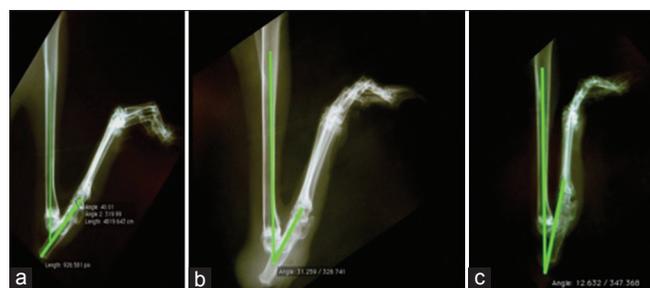


Figure 3: Tibiotalar angle was defined as the angle between the main axis of the tibia and the main axis of the calcaneus. Lateral radiographs showing a 44° tibiotalar angle of a nonoperated leg (a), a 31° tibiotalar angle of a leg submitted to Vulpius Achilles tendon lengthening (b) and 12° tibiotalar angle of a leg submitted to percutaneous Achilles tendon lengthening (c)



Figure 4: Magnetic resonance imaging of nonoperated leg (a), leg submitted to Vulpius Achilles tendon lengthening (b) and leg submitted to percutaneous Achilles tendon lengthening (c). In (b), the arrow shows the scar at the level of the lengthened gastrocnemius fascia. In (c), the arrow shows the scar located between the proximal and distal end of the interrupted Achilles tendon

Table 2: Weight changes in operated rabbits

Type of Surgery (Number of rabbits)	Surgery	Cast removal	1 month	2 months	End of experiment
Total (n=6)	4002±432	3302±505	3465±437	3584±461	3593±362
PATL (n=3)	3993±572	3370±684	3528±592	3663±618	3590±501
VATL (n=3)	4015±304	3200±226	3370±183	3465±198	3598±145
P	>0.05	>0.05	>0.05	>0.05	>0.05

Values are expressed as means±SD (g). SD=Standard deviation, VATL=Vulpius Achilles tendon lengthening, PATL=Percutaneous Achilles tendon lengthening

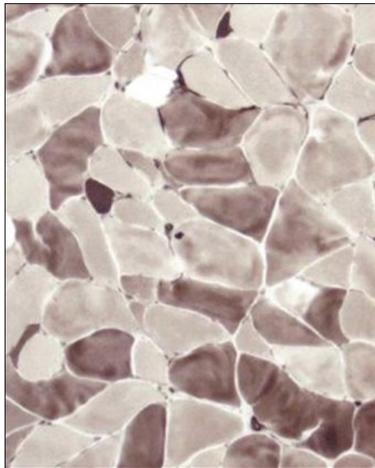


Figure 5: Muscle fibers typed as I, IIA, and IIB based on myosin-ATPase activity: Type I (slow-twitch oxidative; black color) and Type IIA (fast-twitch glycolytic; white color)

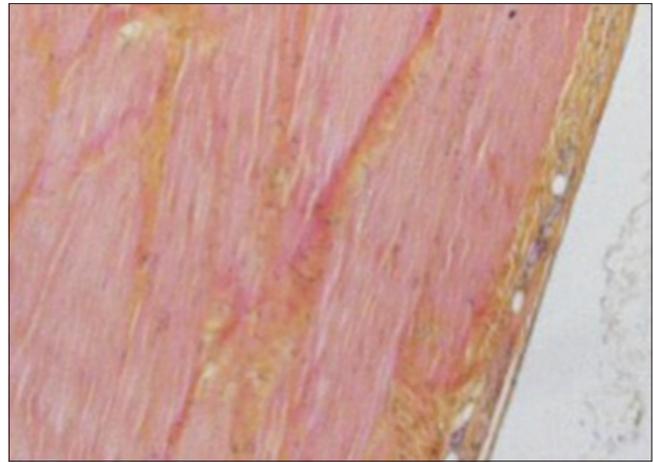


Figure 6: Histology image of the Gastrocnemius muscle and its fascia, before Vulpius Achilles tendon lengthening

Table 3: Clinical findings during postoperative followup

Rabbit	Surgery	Side	Clinical findings	Complications
1	PATL	Right	-	Mild limping Able to jump/ambulate
2	PATL	Bilateral	Local inflammation	Limping
3	PATL	Bilateral	-	Limping
4	VATL	Right	-	None Able to jump/ambulate
5	VATL	Bilateral	-	None Able to jump/ambulate
6	VATL	Bilateral	-	Deceased

VATL=Vulpius Achilles tendon lengthening, PATL=Percutaneous Achilles tendon lengthening

Table 4: Variations in tibiotarsal angle

Rabbits	Surgery	Clinical evaluation (Rx evaluation)	
		Right (°)	Left (°)
1	PATL (right)	0 (12)	42 (45)
2	PATL (bilateral)	0 (11)	3 (13)
3	PATL (bilateral)	0 (12)	4 (12)
4	VATL (right)	14 (31)	44 (45)
5	VATL (bilateral)	10 (32)	14 (31)
6	VATL (bilateral)	Deceased	

Values are expressed in degrees for clinical evaluation (with goniometer) and radiographic evaluation on standard lateral radiographs of the leg and foot. VATL=Vulpius Achilles tendon lengthening, PATL=Percutaneous Achilles tendon lengthening

submitted to VATL varied by about 30° clinically and 15° radiologically [Table 4]. However, rabbits submitted to PATL had poorer functional outcome than rabbits undergoing VATL. In particular, rabbits submitted to PATL developed permanent limp and lost the ability to jump whereas rabbits submitted to VATL remained able to ambulate and jump normally^{25,26} [Table 3].

Evaluations in normal rabbits may at first seem different from observations in children with clubfoot, but in our opinion, they share comparable meaningfulness. In particular, as both PATL and VATL differentially increase TT angle in rabbits, they can be expected to have similar effects in children with congenital clubfoot.^{2,3,7,9,25,26} Anatomy, anatomic relationships of “Gastrocnemius–Achilles tendon–Calcaneus complex”, and some postural attitudes were found to be grossly comparable between rabbits and humans, although differences exist and must be taken into account when using rabbit Achilles tendon as a research model.^{11,14,17,25,26} In particular, the lateral and medial gastrocnemius tendon fibers merge into the Achilles tendon very distally, the soleus makes only a negligible contribution, and a large flexor digitorum superficialis tendon within the tendon sheath rotates dorsally and laterally and runs posterior to the Achilles tendon–calcaneus insertion.⁹ In addition, in rabbits, the flexor digitorum superficialis and medial and lateral gastrocnemius tendon are almost the same size and share the same fascia, which means that PATL lengthens all three tendons together and it may explain limping and gait disturbance in rabbits submitted to PATL^{9,22,26} [Table 1].

In rabbits submitted to PATL, after the Achilles tendon healed, the tension of the muscle–tendon unit never recovered preoperative values, most probably because surgery lengthened the flexor digitorum superficialis tendon and it was performed in subjects that were already skeletally mature. Conversely, in rabbits submitted to VATL, the tension of the muscle–tendon unit remained good enough to allow the rabbits to walk and jump normally.

Note that after surgery (a) the length of bones remains constant; (b) the distance between the proximal and distal insertion of the muscle–tendon unit remains unchanged; and (c) postsurgery, only the ratio between the muscular and tendinous portion of the muscle–tendon unit is modified. Following PATL, the muscular portion of the muscle–tendon unit becomes retracted, and as a result, its

Table 5: Comparison between nonoperated rabbits (control) and rabbits submitted to percutaneous Achilles tendon lengthening and Vulpius Achilles tendon lengthening

Parameters	Nonoperated rabbit/leg	PATL	VATL
Weight loss	None	↓	↓
Functional outcome	Ambulate/jump	↓↓	=
Electromyography	Conduction	=	=
Radiographs (TT)	45°-50°	↓↓↓	↓
Magnetic resonance imaging	Normal muscle-tendon unit	=	=
Histology	Microscopic anatomy	=	=
Histoenzymology	Type IA, IB, and IIB fibers	=	=

TT=Tibiotarsal angle, VATL=Vulpius Achilles tendon lengthening, PATL=Percutaneous Achilles tendon lengthening ↓=mildly diminished; ↓↓=diminished; ↓↓↓=significantly diminished; =: similar

initial length becomes shorter. In particular, the distance between distal insertion of the Achilles tendon and distal end of the muscle belly increases, leading to a reduction of the muscle/tendon ratio of the muscle-tendon unit. We hypothesized that the association between the retraction of the muscle and the lengthening of the tendon results in a reduction of the muscle/tendon ratio, which ultimately leads to a loss of contractile efficiency of the muscle-tendon unit.

The results analyzed carry limitations. First, this was a preliminary study and thus involves a relatively small number of subjects. Second, there are anatomical differences between rabbits and humans, although these differences were taken into account before the study started and anatomical dissection was performed before surgery, which allowed us to note that it was possible to perform both the surgical techniques in rabbits in the same way as usually performed in children. Third, even if humans and rabbits are both plantigrade and pentadactyl, they do not share exactly the same posture; it should however be noted that the orientation of the angle TT is the same.

Conclusions

The Achilles lengthening procedures applied here on the “Gastrocnemius–Achilles tendon–Calcaneus complex” of healthy skeletally mature rabbits, which are anatomically similar to humans, had different outcomes: (1) PATL led to significantly higher tendon lengthening than VATL; (2) PATL led to significantly more reduction in TT angle than VATL; (3) although PATL leads to greater improvement in dorsal or anterior flexion of the rabbit ankle compared to VATL, it tended to negatively impair the ability to ambulate and jump. However, muscle histoenzymology, like electromyographic analysis, did not find significant differences among the two groups, and the surgical interventions applied here to normal rabbits succeeded in amplifying the differences obtained with the same surgical interventions performed in human clubfoot.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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