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Research article

Early Superimposed NMES Training is Effective to Improve Strength and Function Following ACL Reconstruction with Hamstring Graft regardless of Tendon Regeneration

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Abstract
The study aimed at investigating the effects of neuromuscular electrical stimulation superimposed on functional exercises (NMES+) early after anterior cruciate ligament reconstruction (ACLr) with hamstring graft, on muscle strength, knee function, and morphology of thigh muscles and harvested tendons. Thirty-four participants were randomly allocated to either NMES+ group, who received standard rehabilitation with additional NMES of knee flexor and extensor muscles, superimposed on functional movements, or to a control group, who received no additional training (NAT) to traditional rehabilitation. Participants were assessed 15 (T1), 30 (T2), 60 (T3), 90 (T4) and at a mean of 380 days (T5) after ACLr. Knee strength of flexors and extensors was measured at T3, T4 and T5. Lower limb loading asymmetry was measured during a sit-to-stand-to-sit movement at T1, T2, T3, T4 and T5, and a countermovement-jump at T4 and T5. An MRI was performed at T5 to assess morphology of thigh muscles and regeneration of the harvested tendons. NMES+ showed higher muscle strength for the hamstrings (T4, T5) and the quadriceps (T3, T4, T5), higher loading symmetry during stand-to-sit (T2, T3, T4, T5), sit-to-stand (T3, T4) and countermovement-jump (T5) than NAT. No differences were found between-groups for morphology of muscles and tendons, nor in regeneration of harvested tendons. NMES+ early after ACLr with hamstring graft improves muscle strength and knee function in the short- and long-term after surgery, regardless of tendon regeneration.

Key words: Resistance training, semitendinosus, electrical stimulation, knee, rehabilitation.

Introduction

Individuals who underwent ACL reconstruction using semitendinosus and gracilis (STGR) tendon graft show both short- and long-term impairments in hamstring strength following surgery (Beynnon et al., 2002; Heijne and Werner 2010; Nomura et al., 2015; Konrath et al., 2016; Abourezk et al., 2017; Labanca et al., 2020). The quadriceps muscle is also affected by weakness, but the highest impairments have been reported in muscle strength of the knee flexors (Eriksson et al., 2003; Vertullo et al. 2017; Sinding et al., 2020). It is widely documented that weakness of knee flexor muscles in the operated limb is related to some morphological and functional muscle-tendon changes occurring in the semitendinosus and gracilis muscles (Makihara et al., 2006; Åhlén et al. 2012; Snow et al. 2012; Suijkerbuijk et al. 2015; Nomura et al., 2015; Konrath et al., 2016). A significant decrease in volume and length of muscle bellies has been observed, together with a lack of regeneration of the tendons harvested for surgery, which occurs only in a small percentage of patients (Åhlén et al. 2012; Snow et al. 2012; Suijkerbuijk et al. 2015; Konrath et al., 2016). In addition, in the few patients showing regeneration of the tendon, a disomogenous structure of the tendon was observed when compared with the non-operated limb, which featured a larger cross-sectional area, areas of hypervascularity, disorganized architecture, and irregular organization of the collagen fibres (Gill et al., 2004).

It is not clear which factors account for the regeneration or the lack of regeneration of the tendon. However, it is well known that tendon structure is strongly influenced by the mechanical loading that is generated by muscle belly activation. It has been shown that strength training is beneficial for the regeneration of the harvested tendons (Enwemeka, 1992), and that collagen synthesis and protein synthesis have the same time course of changes, thus showing that muscle and tendon adaptations are coordinated in response to exercise (Miller et al., 2005). At the same time, it has also been shown that tendon structure is negatively affected by the lack of muscle belly activity, as for example during immobilization, which leads to a lower tendon stiffness (Couppé et al., 2012). Thus, an early exercise intervention for the muscles involved in ACL reconstruction should be beneficial in terms of muscle belly function in the short term, and whole muscle-tendon unit in the long term. However, it is extremely difficult to target the muscles involved in surgery through resistance training interventions due to post-surgical neural inhibition, which results in patients being unable to voluntarily control muscle activation (Hopkins and Ingersoll, 2000). When a muscle is not activated during training, inter-muscular agonistic compensation may occur, as shown by a hypertrophy of the biceps femoris and semimembranosus muscles in patients with harvested STGR tendons, which are overloaded to compensate for the lack of activity of semitendinosus (Konrath et al., 2016). It should also be considered that training is effective when an adequate intensity is selected, but it is not safe to perform high intensity strength training early after surgery.

Neuromuscular electrical stimulation has been used

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in rehabilitation following ACL reconstruction either as single treatment or in combination with voluntary exercise as it allows a selective strengthening of the muscles that are affected by neural inhibition (Snyder-Mackler et al., 1994; Delitto et al., 1998; Fitzgerald et al., 2003; Lepley et al., 2015). However, full recovery of muscle strength was not achieved in most cases and patients showed deficits for years after surgery. Only recently, Labanca et al. (2018) have demonstrated the effectiveness of a novel approach, based on superimposing neuromuscular electrical stimulation to voluntary dynamic movements, which allows high-intensity resistance training on the quadriceps muscle during the early phase following surgery without overloading the knee joint. The authors, for the first time, have shown that using this approach during the first two months following ACL surgery with bone-patellar-bone graft resulted in a fully symmetrical recovery of quadriceps muscle strength at six months. This approach has the potential to be adapted for strengthening the hamstrings following ACL reconstruction with STGR tendon graft. Moreover, as the tendon structure is strongly influenced by the mechanical loading that is generated by muscle belly activation, there are good reasons to believe that this approach may also contribute to tendon regeneration.

Therefore, the objective of this randomized controlled study was to investigate the effects of an early intervention based on neuromuscular electrical stimulation superimposed on functional movements, in addition to standard rehabilitation, on knee flexor muscle strength, functional performance, and thigh muscle-tendon unit morphology following ACL reconstruction using STGR tendon graft. It was hypothesised that this novel training intervention would have been effective in improving muscle strength, knee function, and thigh muscle-tendon unit morphology, in comparison to a standard rehabilitation with no additional training intervention.

Methods

Participants and intervention training protocol

Sixty-four ACL reconstructed subjects who received a hamstring tendon graft and underwent post-surgical rehabilitation at the same rehabilitation centre were assessed for eligibility.

Inclusion criteria were as follows:

- Aged between 18 and 50 years;
- ACL reconstruction using semitendinosus and gracilis tendon graft;
- Physical activity level: Tegner Activity Scale level from 5 to 9 (Tegner and Lysholm, 1985);
- Range of motion (ROM) 15 days after surgery: 0°-90°.

Exclusion criteria were as follows:

- Pathologies or conditions in which electrical stimulation was contraindicated (e.g. pacemaker, pregnancy, epilepsy);
- Neurological disorders;
- History of muscle and tendon injuries in lower limbs;
- Knee pain and swelling affecting exercise performance two weeks after surgery;
- Lack of adherence to the prescribed rehabilitation.

Forty-six patients met the inclusion/exclusion criteria and were recruited for this study. A flow-chart illustrating patient enrolment is represented in Figure 1. Twelve patients dropped out of the study; thus, the analysis was carried out on 34 participants, 20 males and 14 females.

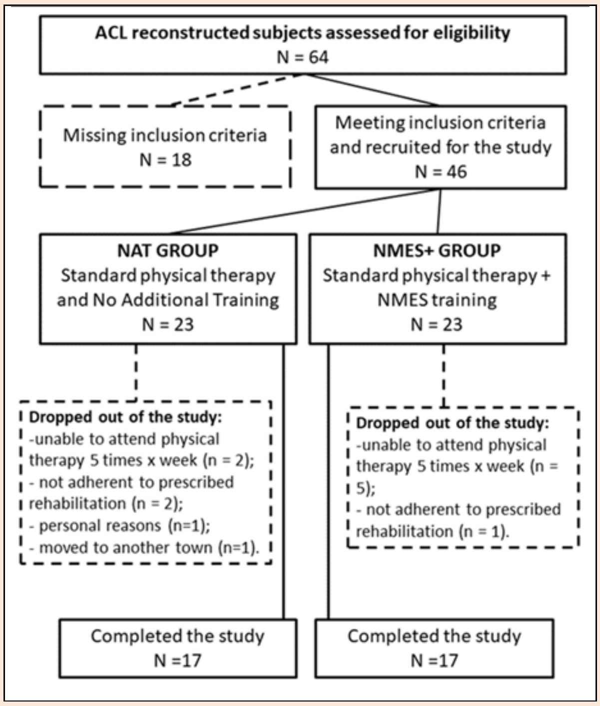

Figure 1. Participants' enrolment flow-chart.

Participants were assigned by means of randomization for age, gender and Tegner level to one of the two cohorts: the neuromuscular electrical stimulation superimposed on movement group (NMES+) and the no additional training group (NAT), which served as a control group. The NMES+ group was composed of 17 participants, 10 males and 7 females (age: 34.6 ± 8.7 years; stature: 1.70 ± 0.06 m; body mass: 66 ± 9 kg; Tegner level: 7.1 ± 0.9), and similarly, the NAT cohort was composed of 17 participants, 10 males and 7 females (age: 34.3 ± 9.9 years; stature: 1.74 ± 0.01 m; body mass: 74 ± 11 kg; Tegner level: 7.1 ± 0.9).

The same standardized postoperative rehabilitation protocol was administrated under supervision by the same team of physical therapists 5 days a week, as previously described (Rocchi et al. 2020). Patients in the NMES+ group received an additional training protocol based on a number of sit-to-stand-to-sit (STSTS), squat (SQ) and step-up-step-down (STEP) tasks with the superimposition of neuromuscular electrical stimulation. Training lasted from Day 15 to Day 60 and consisted of 5 sessions per week. The neuromuscular electrical stimulation was given via a wireless portable battery-powered stimulator (Chattanooga Wireless Professional), which produced a rectangular biphasic pulse in response to a voluntary contraction of the muscle. Self-adhesive electrodes (Compex Dura-Stick plus; 5x5 cm) were placed on the operated limb over the motor points of the semitendinosus, vastus lateralis, vastus medialis and rectus femoris muscles. Motor points were identified in accordance with the electrical stimulator user's guide. Two frequencies of stimulation were used, 35 and 50 Hz, which were alternately applied at each session in accordance with a previous study (Labanca et al. 2018). The intensity of stimulation was increased by the trainer during each exercise repetition throughout the session, in accordance with patient tolerance, to maximise motor unit recruitment (Maffiuletti, 2010; Borzuola et al. 2020). In addition, the intensity was selectively increased for each muscle to guarantee a homogeneous distribution of the exercise load. Patients were encouraged to voluntarily activate their muscles throughout the duration of movement. The maximum intensity given by the stimulator was 120 mA.

Quadriceps muscle was stimulated during the STSTS task. Participants were asked to sit and maintain a 90° knee flexion angle. The stimulation lasted 8 seconds and was initiated by the quadriceps muscle contraction, as set in the electrical stimulator. Patients were asked to contract their quadriceps and, after the onset of the stimulation, to perform a sit-to-stand movement (concentric phase) followed by a stand-to-sit movement (eccentric phase) in 8 seconds, and then sitting to rest for 8 seconds, creating a duty cycle of 16 seconds.

The semitendinosus muscle was stimulated during the SQ task for the first two weeks of training, and then it was stimulated during the STEP task from the third to the eighth week. During the SQ, participants were asked to stand up, which was the starting position, and then to squat down to approximately an 80° knee flexion angle (concentric phase) and finally to return in the upright position (eccentric phase). The stimulation lasted 8 seconds. Patients were asked to perform the SQ in 8 seconds, and then to sit, resting for 8 seconds, thus creating a duty cycle of 16 seconds.

During the STEP task participants were asked to stand up with the foot of the operated limb, which was positioned on a 20 cm step. The foot of the operated limb was maintained in contact with the step for the duration of movement. Participants were asked to step up with the operated limb reaching full knee extension (starting position), then to step down (concentric phase), reaching the floor with the foot of the non-operated limb, and finally to step up again (eccentric phase). The stimulation lasted 8 seconds. Patients were asked to perform the STEP in 8 seconds, and then return to the starting position to rest for 8 seconds, creating a duty cycle of 16 seconds. A bar adjustable in height was positioned in front of participants for hand support during the STEP movement. Participants could modify the starting and ending knee angles during training if they felt pain or excessive discomfort to the operated knee joint. The duration of the concentric and the eccentric phases of all tasks varied over the intervention, along with the number of sets and repetitions throughout the programme. A detailed description of the NMES+ training protocol is reported in Table 1.

Before each NMES+ session, patients were asked to warm up on an exercise bicycle at low resistance for 10 minutes. During the first week of training (from the 15th to the 20th day), if patients were not able to perform cycling movements, a passive and an active knee joint mobilization was supervised by a physical therapist.

Table 1. NMES+ training description over the six weeks of the intervention.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Task ST	SQ	SQ	STEP	STEP	STEP	STEP
Task Quad	-	STSTS	STSTS	STSTS	STSTS	STSTS
Sets x Reps	3 x 6	3 x 8	3 x 8	3 x 10	3 x 12	3 x 12
	-	2 x 8	2 x 8	2 x 10	2 x 12	3 x 12
Con / Ecc	4 / 4	4 / 4	4 / 4	2 / 6	2 / 6	2 / 6
	-	4 / 4	2 / 6	2 / 6	2 / 6	2 / 6

Task ST = task used to train the semitendinosus muscle: SQ = squat; STEP = step-up-step-down. Task Quad = task used to train the quadriceps muscle: STSTS = sit-to-stand-to-sit. Sets x Reps = number of sets and repetitions for each task. Con /Ecc = duration (s) of the concentric and eccentric phases of each task.

Patients in the NAT group received the standard rehabilitation programme as previously described with no additional training.

The therapists who performed the additional NMES rehabilitation were aware that patients were involved in a research study. The authors who performed the assessments, the MRI and data analysis were blinded on patients' allocation.

The study was approved by the Ethics Committee of the University of Rome La Sapienza (prot. n. 293/19), was carried out in accordance with the Declaration of Helsinki and all participants signed an informed consent form.

Sample size and statistical power

Sample size was a priori calculated with a significance level of $\alpha = 0.05$ and a power of 95% based on data of a preliminary pilot investigation on 6 patients who were randomly assigned to one of the 2 groups (NMES+ and NAT). Effect size was calculated based on the mean limb symmetry index (LSI) of peak forces recorded during maximal voluntary isometric contraction of knee flexor muscles 60 days after surgery at 90°, which was 74.8% for the first group, and 64.4% for the second. A minimum of 17 patients for each group was required for the study. Additional patients were recruited to allow for dropouts.

Assessments and data analysis

Assessments were performed 15 days (T1), 30 days (T2), 60 days (T3), 90 days (T4) and in the long term after surgery (T5). For the long-term assessment, patients were only assessed if they had returned to their pre-injury activity level for at least two months. Mean time of the T5 assessment was 380 days. A timeline representation of the assessments is reported in Figure 2.

Morphological examination of thigh muscles and tendons

Morphological examination via MRI of thigh muscles and tendons was performed at T5. Axial Dixon sequences were acquired using a 1.5 T MRI scanner (Ingenia; Philips, Amsterdam, The Netherlands) from the level of the iliac crest to the tibial tuberosity spanning both legs while the subject lay supine in the scanner. The images were acquired in 2 stations with 230 slices per station and a 10% overlap between stations. Slice thickness was 2.4 mm with a 0.5 mm

Figure 2. On the right, coronal MRI of right (R) and left (L) limbs with highlighted semitendinosus muscle in one of the participants with ACL reconstruction of the right limb. On the left, transverse MRI with highlighted distal semitendinosus insertion. No regeneration was observed for the harvested tendon.

intersection gap, repetition time (TR) was 4.1milliseconds, echo time 1 (TE 1) was 5.9 milliseconds, and echo time 1.92 (TE 2) was 3.9 milliseconds. Both TEs were collected when water and fat were in phase and out of phase. The voxel size was 1.1 - 1.1 - 2.4 mm, and the field of view (FOV) was 450 - 288 - 279 mm. The Fast Field Echo (FFE) images provided excellent visibility of the muscle for manual segmentation. Axial proton density (PD) 2-dimensional turbo spin-echo sequences were also acquired from the level of the mid-thigh to below the tibial tuberosity spanning both legs. The images were acquired in 1 station of 80 slices with a slice thickness of 3.2 mm and a 0.5 mm intersection gap; TR was 3954 milliseconds and TE was 30 milliseconds. The voxel size was 0.8 - 0.8 mm, and the FOV was 380 - 220 - 296 mm. The PD images provided excellent visibility of the tendons for manual segmentation.

Image analysis was performed by two experienced musculoskeletal radiologists using the software OsiriX (version 11.0, Pixmeo Sarl, Geneva, Switzerland). Tendons of the donor muscles were first inspected to assess the regeneration and morphology. Tendon regeneration was confirmed if the tendon was visible below the musculo-tendinous junction as in previous literature (Konrath et al. 2016). If the tendon had regenerated, then it was assessed for morphology. The major diameter of the tendon in the mediolateral direction for each axial slice was recorded to express tendon width. The distal musculo-tendinous junction and the tibial insertions were identified and marked to calculate the tendon length as previously described (Ilahi et al. 2018). Figure 2 shows a typical example of MRI in one of the participants, who showed no regeneration in the harvested tendons.

Volume of the vastus medialis (VM), vastus lateralis (VL), vastus intermedium (VI), rectus femoris (RF), sartorius (SA), gracilis (GR), semitendinosus (ST), semimembranosus (SM) and biceps femoris (BF) muscle bellies were calculated. The margins of every single muscle were manually traced in each axial slice. The margins of the axial slices were used to represent a 3D model of each muscle. Briefly, for each muscle, cross-sectional areas were fitted with a cubic spline and plotted against femur length. Muscle volumes were calculated as the area under the curve using the measured and splined data points as in previous studies (Morse et al., 2007; Kulas et al., 2018). For the ST and GR donor muscles, the length of muscle belly was calculated whether regeneration occurred or not. The length of the muscle belly was calculated from a line drawn to connect the centroid of each slice for each muscle. Tendon length and width, muscles belly volume and length were calculated for both limbs and were used for further analysis.

Muscle strength assessment

Muscle strength was assessed by means of maximal isometric voluntary contractions (MVIC) and isokinetic tests. MVIC of the knee extensor muscles at a knee angle of 30° and 90° and MVIC of the knee flexor muscles at a knee angle of 90° were carried out in both limbs at T3, T4 and T5. At T5, knee flexor muscles were assessed also at a knee angle of 60°, which was not performed in earlier assessments to avoid muscle injuries. During the assessment, participants were seated on a leg-extension machine (Technogym, Forli-Cesena, Italy) for the knee extension MVIC and on a leg-curl machine (Technogym, Forli-Cesena, Italy) for the knee flexion MVIC. Patients were fastened using three crossing belts on both machines. Muscle force was recorded using a load cell connected to a computerized system unit (MuscleLab, Bosco-System Technologies, Rieti, Italy). The maximal voluntary isometric contraction task consisted of a progressive increase to a maximum force exerted by the leg muscles. Participants were able to follow their performance on a computer screen and were verbally encouraged to achieve their maximum and to maintain that maximum for at least 2 seconds before relaxing. Maximal voluntary isometric contraction was calculated as the largest 1-second average reached within any single force recording.

Peak forces exerted by each limb were recorded. Side-to-side symmetry was quantified for peak forces using the limb symmetry index, which was calculated as the ratio between the involved and uninvolved limb expressed as a percentage. Absolute force was calculated by normalizing peak forces recorded during all maximal voluntary isometric contractions to the body weight of each participant. The side-to-side symmetry and absolute force values were used for further analysis.

Functional performance assessment

Functional performance was assessed at T1, T2, T3, T4 and T5 by means of a STSTS movement and a countermovement jump (CMJ) which were performed on two adjacent force platforms.

The STSTS was performed at T1, T2, T3, T4 and T5. The sit-to-stand-to-sit task consisted of rising from a seat as fast as possible and sitting down as fast as possible. The height of the seat was adjusted at each assessment to obtain a 90° angle at the knee joint. Participants were asked to keep their trunk in a vertical position, their arms held across the chest, and their feet shoulder-width apart. Participants were verbally instructed to stand up as fast as possible and maintain the upright position for 5 seconds and then to sit down as fast as possible. The CMJ was performed at T4 and T5. Participants were asked to stand in an upright position and maintain their hands on their hips during performance of the CMJ to minimize the influence of the upper limbs. They were asked to quickly squat with knees flexed to approximately 90° and then jump immediately as high as possible without pausing.

Ground reaction forces of STSTS and CMJ were measured by means of two, six-component force platforms (KISTLER, model 9281 B; Winterthur, Switzerland; 100-Hz sampling frequency), which were positioned one below each foot. Vertical components of the ground reaction force were filtered offline using a digital, low-pass, second-order, Butterworth filter with a cutoff frequency set at 15 Hz. Signals from the two force platforms were summed. The STSTS peak forces of the sit-to-stand phase and the stand-to-sit phase were analyzed in accordance with previous studies (Laudani et al., 2014; Baumgart et al., 2015; Labanca et al., 2016). For the CMJ, the signal was then processed according to previous studies (Davies and Rennie, 1968; Laudani et al., 2013). Briefly, the vertical velocity of the centre of mass (CoM) was calculated from the time integration of the instantaneous acceleration. The eccentric and concentric phases (EccP and ConP) of the push-off phase of the jump were identified from vertical velocity of the jump. EccP was identified from the downward movement (negative velocity) of CoM, while ConP from the upward movement of CoM (positive velocity). Peak force recorded during both eccentric and concentric phases was used for further analysis. Absolute peak forces of STSTS, EccP and ConP were used to quantify the side-to-side symmetry using the limb symmetry index (Laudani et al., 2014; Labanca et al., 2018).

Statistical analysis

Descriptive statistics were used to summarize demographic data and the Shapiro-Wilk test was used to test the distribution of all variables. A two-way analysis of variance (ANOVA) for repeated measures was conducted to investigate the effect of rehabilitation and time on all the assessed variables. A one-way ANOVA was used to analyse the differences between the two groups for MVIC of knee flexor muscles at 60° and MRI parameters at T5. A Mann-Whitney test was used to investigate the differences between the two groups for tendon regeneration. An analysis of covariance (ANCOVA) was conducted to assess the effects of NMES+ and standard rehabilitation on all variables, accounting for the gender of the participants. An ANCOVA was also conducted to assess the effects of NMES+ and standard rehabilitation on the assessments performed at T5, after accounting for tendon regeneration. Finally, to investigate the predictive variables of the tendon regeneration, a binary logistic regression analysis was performed an all data using tendon regeneration as a dependent variable. Student's t-test was used to locate all the significant differences. A significance level of $p < 0.05$ was adopted. The analyses were performed using SPSS version 20.0 (SPSS, Inc, Chicago, IL).

Results

Regeneration and morphology of harvested tendons and muscles

Regeneration of the semitendinosus tendon was observed in 6 participants of the NMES+ group and 8 of the NAT group. Regeneration of the gracilis tendon was observed in 7 participants of the NMES+ group and 3 participants of the NAT group. Regenerated tendons of the semitendinosus muscle showed no significant differences between the two groups in width (NMES+ = 4.7 ± 2.3mm; NAT = 3.8 ± 1.8mm) and length (NMES+ = 158.3 ± 30.6 mm; NAT = 155.0 ± 33.4 mm), but the regenerated tendon was significantly longer than the contralateral limb in both groups (NMES+ 158.3 ± 30.6 mm vs. 121.7 ± 27.9 mm; $p < 0.01$); NAT (155.0 ± 33.4 mm vs. 106.3 ± 10.6 mm; $p < 0.01$). The regenerated tendon was not significantly different from the contralateral limb in width (NMES+: operated 4.7 ± 2.3mm, contralateral 4.0 ± 1.3 mm; NAT: operated 3.8 ± 1.8 mm, contralateral 4.6 ± 0.7 mm).

Regenerated tendons of the gracilis muscle in both groups showed no between-limb differences in width (NMES+: operated 3.9 ± 1.7 mm, contralateral 3.8 ± 0.7 mm; NAT: operated 3.1 ± 2.0 mm, contralateral 3.0 ± 0.0 mm) and length (NMES+: operated 111.4 ± 37.2 mm, contralateral 98.6 ± 10.7 mm; NAT: operated 66.7 ± 20.8 mm, contralateral 80.0 ± 26.5 mm), and no between-group differences.

None of the variables assessed in this study were a predictor of tendon regeneration, as shown by the regression analysis model which failed to predict tendon regeneration (β = 0.251; $p = 0.61$).

For participants showing a regeneration of the semitendinosus tendon, no significant differences were observed between the two groups for muscle belly volume (NMES+ = 120.7 ± 82.5 mm^3; NAT = 111.4 ± 53.2 mm^3) and length (NMES+ = 244.2 ± 46.7 mm; NAT = 233 ± 29.3 mm). In the NAT group there was a significant difference in the operated compared with the non-operated limb for muscle belly length (233 ± 29.3 mm vs. 294.9 ± 29.3 mm; $p < 0.01$) and volume (111.4 ± 53.2 mm^3 ± 182.1 ± 75.6; $p < 0.01$).

For patients showing no regeneration of the tendon, no between-groups differences were observed, but the harvested semitendinosus when compared to the contralateral limb had a lower volume in NMES+ group (82.2 ± 49.2 mm^3 vs. 138.1 ± 62.6 mm^3; $p < 0.01$) and NAT group (53.0 ± 19.2 mm^3 vs. 94.8 ± 45.3 mm^3; $p < 0.01$), and a lower length in NMES+ group (206.1 ± 42.0 vs. 277.0 ± 44.1; $p < 0.01$) and NAT group (216.4 ± 36.5 vs. 265.0 ± 68.9; $p < 0.01$).

Participants showing a regeneration of the gracilis tendon in the NMES+ group had in the operated compared to the non-operated limb a lower volume (66.7 ± 27.7 mm^3 vs. 84.9 ± 23.3 mm^3; $p < 0.05$) and a lower length (281.3 ± 25.8 mm vs. 314.7 ± 22.9 mm; $p < 0.05$) of gracilis muscle belly. While participants who did not show a regeneration had in the operated compared to the non-operated limb a lower length (243.2 ± 52.9 mm vs. 296.8 ± 17.4 mm; $p < 0.05$) of gracilis muscle belly.

Participants who did not show a regeneration of gracilis tendon in the NAT group had in the operated compared to the non-operated limb a lower volume (57.6 ± 31.6 mm^3 vs. 91.7 ± 50.3 mm^3; $p < 0.05$) and a lower length (239.3 ± 34.6 mm vs. 298.4 ± 51.2 mm; $p < 0.05$) in gracilis muscle belly.

Morphology of the other thigh muscles

No differences between the two groups were observed for muscle belly volume of all muscles, but the ANCOVA showed that gender had an effect, with females having lower muscle belly volume in both limbs. Mean values and standard deviations of the volumes of all muscle bellies analyzed in male and female patients of each group, with significant differences after the post-hoc analysis are represented in Figure 3 (knee flexor muscles) and Figure 4 (other muscles).

Muscle strength

Significant differences were identified between the two groups for muscle strength normalized by body weight of the operated limbs. Mean values with standard deviations and significant differences following the post-hoc analysis are reported in Table 2. Participants in the NMES+ group demonstrated a higher muscle strength at T3 during the MVIC of knee extensor muscles at 30°, at T4 for all the measurements, and at T5 for the MVIC of knee extensor muscles at 30° and knee flexor muscles at 90°. There were also significant differences between male and female participants.

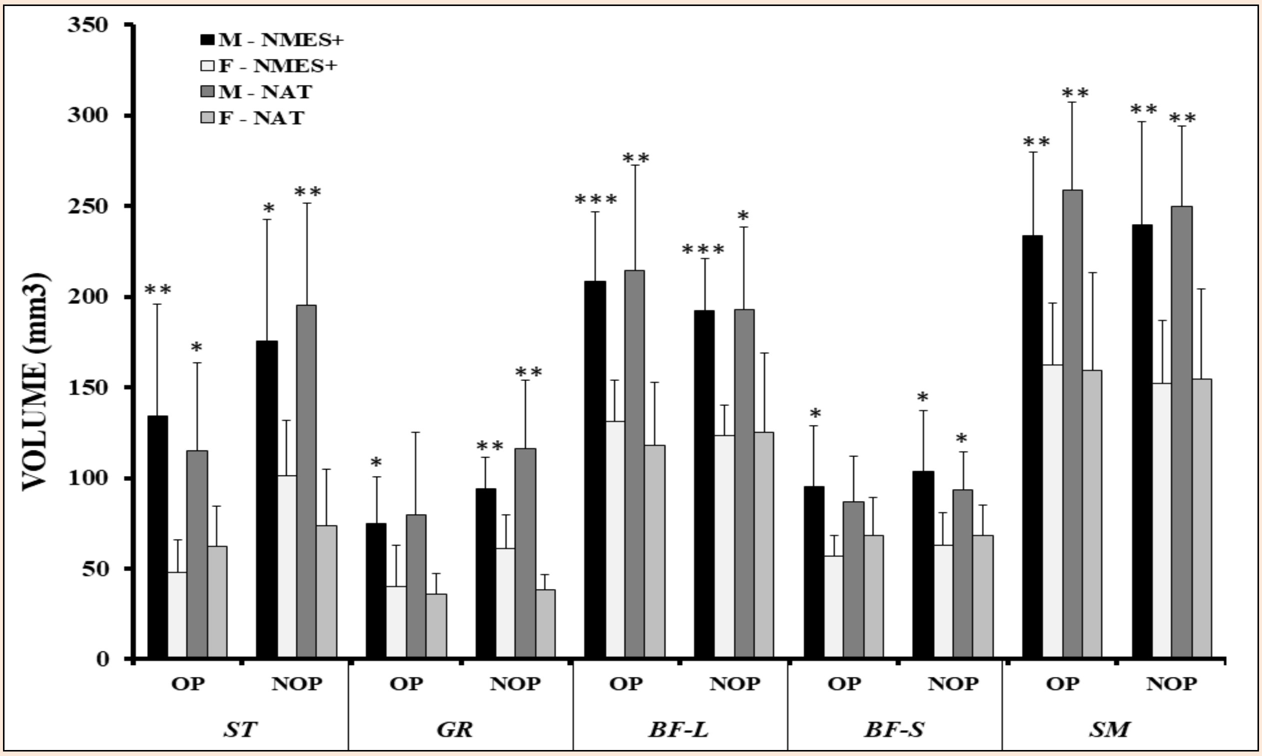

Figure 3. Muscle volumes of semitendinosus (ST), gracilis (GR), long head of biceps femoris (BF-L), short head of biceps femoris (BF-S) and semimembranosus (SM) in the operated (OP) and non-operated (NOP) limb, in male and female participants of each group. Significant differences from female participants: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

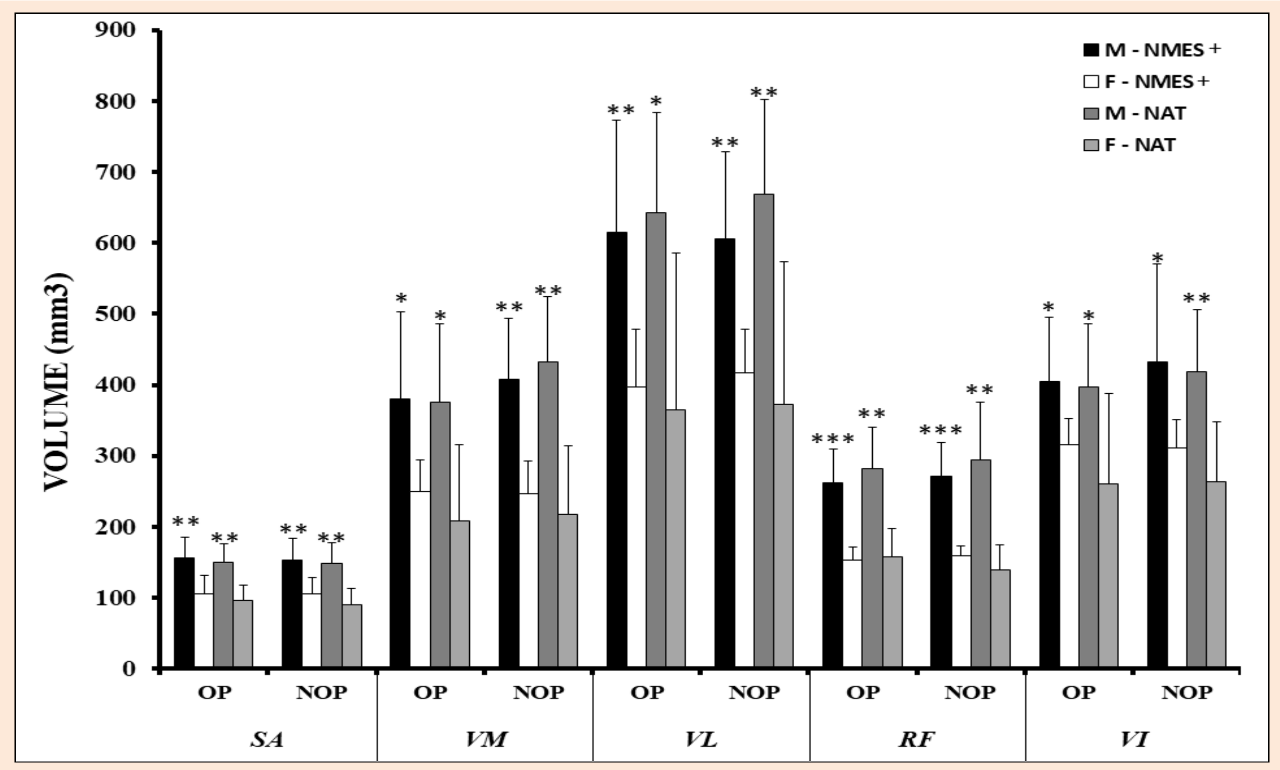

Figure 4. Muscle volumes of sartorius (SA), vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF) and vastus intermedius (VI) in the operated (OP) and non-operated (NOP) limb, in male and female participants of each group. Significant differences from female participants: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 2. Mean values and standard deviations of peak forces normalized by body weight of the operated limb recorded during the MVICs of knee extensor muscles at 30° (Ext 30°) and 90° (Ext 90°), and knee flexor muscles at 60° (Flex60°) and 90° (Flex 90°), at T3, T4 and T5. Values are reported for males and females of each group, and for each whole group (NMES+ and NAT).

			MVIC - Operated Limb / body weight								
			T3			T4			T5		
			Ext 30°	Ext 90°	Flex 90°	Ext 30°	Ext 90°	Flex 90°	Ext 30°	Ext 90°	Flex 90°
NMES+	Males	mean	0.73[a]	0.65[a]	0.44[a]	0.85	0.78	0.53[a]	1.03[a]	0.95[a]	0.62
		st. dev.	0.17	0.19	0.13	0.31	0.34	0.15	0.25	0.26	0.17
	Females	mean	0.47[a]	0.37[a]	0.28[a]	0.57	0.58	0.33[a]	0.61[a]	0.73[a]	0.47
		st. dev.	0.18	0.08	0.09	0.18	0.09	0.09	0.35	0.11	0.10
	Group	mean	0.62*	0.53	0.37	0.74*	0.70*	0.45*	0.85*	0.85	0.56
		st. dev.	0.21	0.21	0.14	0.29	0.29	0.16	0.36	0.23	0.16
NAT	Males	mean	0.56[a]	0.57[a]	0.33	0.64[a]	0.61[a]	0.38[a]	0.76[a]	0.79[a]	0.56
		st. dev.	0.24	0.19	0.16	0.27	0.26	0.14	0.24	0.22	0.13
	Females	mean	0.34[a]	0.33[a]	0.22	0.37[a]	0.33[a]	0.23[a]	0.43[a]	0.59[a]	0.38
		st. dev.	0.10	0.11	0.11	0.14	0.16	0.04	0.09	0.14	0.08
	Group	mean	0.47*	0.47	0.28	0.51*	0.48*	0.31*	0.52*	0.72	0.49
		st. dev.	0.22	0.20	0.15	0.25	0.25	0.13	0.38	0.22	0.14

* significant difference between the two groups (NMES+ and NAT), [a] significant difference between males and females.

The ANOVA also showed an effect of time on MVICs results. In the NMES+ group there was a significant increase of LSI of muscle strength of knee extensors and flexors at 90° between T3 and T4, and for all the measurements between T4 and T5 (Figure 4). Muscle strength of the operated limb significantly increased between T3 and T4, and T4 and T5 for knee extensor muscles at 90°. A significant increase for flexor muscles at 90° was found between T4 and T5. In the NAT group significant increases of the LSI of all measurements were found only between T4 and T5. Strength of the operated limb significantly increased for extensors and flexors at 90° between T4 and T5.

Figure 5 shows mean values with standard deviations of the limb symmetry index of MVICs at T3, T4 and T5. When compared with NAT group, participants in the NMES+ group showed a significantly higher symmetry during MVIC of knee extensor muscles at 30° at T3, and of knee flexor muscles at T4. At T5 they showed higher symmetry in all the assessments when compared with NAT participants.

Functional performance

The mean values and standard deviations of the LSI of peak forces recorded during the sit-to-stand, stand-to-sit, the eccentric phase of the CMJ, and the concentric phase of the

CMJ, along with the significant differences between the two groups after the post-hoc analysis are presented within Table 3. The ANOVA showed an effect of time on all data. In the NMES+ group there was a significant increase in the LSI of the sit-to-stand and the stand-to-sit between T1 and T2, and T2 and T3. For the NAT group, a significant increase in the LSI of the sit-to-stand was found between T2 and T3, and T4 and T5. For LSI of the stand-to-sit, a significant increase was found only between T4 and T5. LSI of both phases of the CMJ significantly increased between T4 and T5 in the NMES+ group, while no significant increases were found in the NAT group.

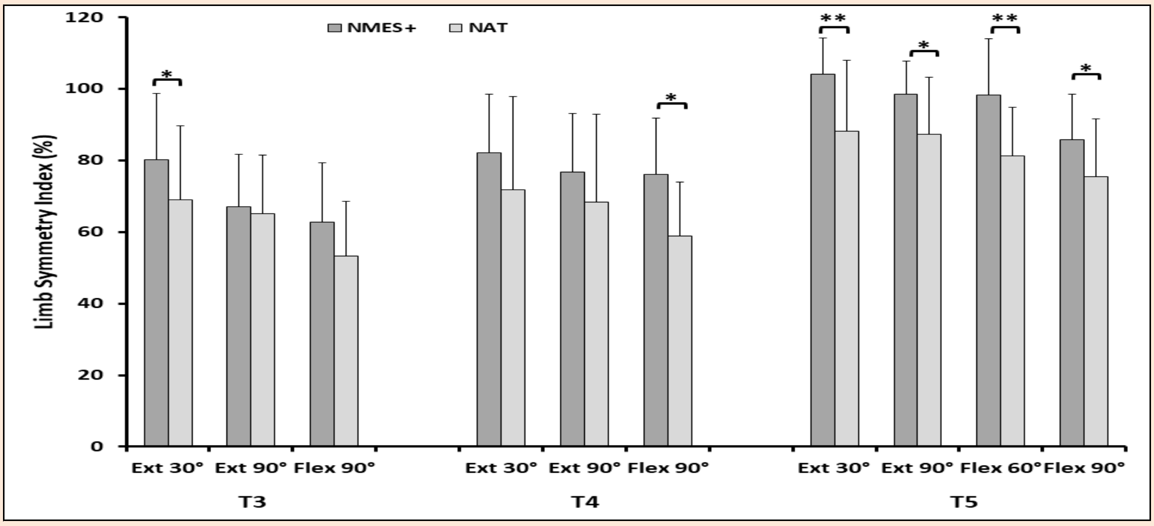

Figure 5. Mean values and standard deviations of the limb symmetry index of MVIC of knee extensor muscles at 30° (Ext 30°) and 90° (Ext 90°), and knee flexor muscles at 90° (Flex 90°) and 60° (Flex 60°), at T3, T4 and T5. * $p < 0.05$, ** $p < 0.01$.

Table 3. Limb symmetry index of peak forces recorded during the sit-to-stand, the stand-to-sit, the eccentric phase of the CMJ, and the concentric phase of the CMJ.

		LIMB SYMMETRY INDEX													
		Sit To Stand					Stand To Sit					CMJ ecc		CMJ con	
		T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T4	T5	T4	T5
NMES+	mean	58.6	71.9	89.8*	90.0*	96.9	51.1	83.0*	89.2*	89.7*	94.0*	85.2	96.7*	86.9	95.3*
	st. dev.	15.4	9.6	14.3	12.1	11.4	14.4	12.9	13.0	13.4	9.7	10.4	9.6	14.4	6.4
NAT	mean	55.9	66.0	80.9*	80.1*	91.7	56.4	67.0*	77.7*	77.2*	86.9*	79.3	86.0*	82.3	88.7*
	st. dev.	13.1	13.9	12.8	15.0	9.3	13.4	21.0	14.0	18.0	7.7	11.8	8.8	9.0	9.5

* significant difference between NMES+ group and NAT group.

Discussion

The main result of this study was that a novel training intervention based on neuromuscular electrical stimulation superimposed on functional movements during the early phase following ACL reconstruction with STGR tendon graft, as an additional treatment to standard rehabilitation, was more effective in improving muscle strength and knee function, both in the short and in the long term after surgery, than a standard rehabilitation with no additional training intervention. Interestingly, these adaptations occurred regardless of tendon regeneration of the donor muscles.

Increasing the intensity of strength training with traditional physical therapy exercises is difficult in the first two months after surgery as it is impossible to overload the knee joint with heavy loads, which are essential to increase muscle strength. The superimposition of NMES allowed an increase of the intensity of muscle activation at each repetition of easy functional tasks (squat, sit-to-stand, stepping) which can be performed in the early phase after surgery by all the patients but do not normally require high intensity muscle activations. In addition, both the hamstring muscles, which were harvested for ACL graft, and the quadriceps muscle are affected by atrophy and inhibition (Hopkins and Ingersoll, 2000). Thus, without electrical stimulation it would have been impossible to reach high levels of muscle activation. In addition, the lack of activation of the target muscles leads to inter-agonistic muscle compensations, and to an overload of the passive joint structures, which may be mechanically damaged (Arokoski et al. 2008).

The benefits of NMES+ training were apparent following the first assessment, which was performed 15 days after the beginning of training (i.e., 30 days after surgery). Participants of the NMES+ group showed a higher symmetry in lower limb loading was found in NMES+ compared to NAT during the stand-to-sit movement, which is comparable to the results of Labanca et al. (2018).

This result was confirmed during the second assessment, which was carried out at the end of the NMES+ training intervention (i.e., 60 days after surgery), and at this time point NMES+ participants also showed a higher symmetry than the NAT group during the sit-to-stand movement. In addition, 60 days after surgery it was possible to safely assess muscle strength for the first time. Participants in the NMES+ group showed higher absolute muscle strength and higher symmetry of the knee extensor muscles in the operated limb at 30° than in the NAT group. To the best of the authors' knowledge, there are no strength training interventions based on high intensity muscle contractions starting as early as fifteen days after surgery, except Labanca et al. (2018) who have recently carried out a similar early strength training intervention following ACL reconstruction with patellar tendon graft. However, it is difficult to compare the results of the two studies because of the differences in impairment of quadriceps muscle function that relate to the type of graft. In both studies, the early recovery of quadriceps muscle strength was accompanied by an improvement in knee function. The early reversal of muscle inhibition during the early phases following surgery is essential for two main reasons: first, molecular and neural alterations lead to muscle atrophy following ACL injury injury (Konishi et al., 2002; Jackman and Kandarian, 2004), and the longer a muscle remains in an atrophy condition the longer it takes for atrophy reversal; second, the early atrophy reversal allows training at higher intensities during the intermediate and advanced phases of rehabilitation. Moreover, an increase in strength leads to a decrease in abnormal forces on passive joint structures (Arokoski et al., 2008), which in turn reduces joint swelling. Less swelling of the knee joint means, avoiding further inhibitory reflexes on the quadriceps muscle (Rice and McNair, 2010).

Interestingly, one month after the end of NMES+ treatment (i.e., 90 days after surgery), participants in the NMES+ group maintained higher thigh circumference, lower knee circumference, higher knee joint range of motion of the operated knee and a higher symmetry in lower limb loading during the sit-to-stand and stand-to-sit tests than participants of the NAT group. This trend is confirmed by the long-term assessment, i.e., at about one year after surgery, whereby the muscle strength of the operated limb for both knee extensor and flexor muscles remained higher in the NMES+ group compared to the NAT group. This observation is supported by the results of previous studies showing that an early gain in strength and function is predictive of long-term outcomes (Labanca et al., 2016; Hanada et al., 2019). It is likely that the earlier recovery of muscle strength and symmetrical lower limb loading allowed for the sustention of higher training loads during the subsequent phases of rehabilitation, thus leading to improved outcomes from the end of the NMES+ training onwards. From a clinical perspective, returning to sport activities with a higher and more symmetrical between-limbs muscle strength leads to a decrease in the risk of reinjury (Grindem et al., 2016) and of longer-term knee osteoarthritis (Patterson et al., 2020).

The higher increase in strength of the quadriceps muscle in the NMES+ group than in the NAT group might also explain the higher improvements in other functional outcomes such as the sit-to-stand and the concentric phase of the CMJ. It is interesting to note that participants in the NMES+ group also showed higher symmetry than NAT participants during the stand-to-sit test and during the eccentric phase of the CMJ. These latter tests require high levels of strength and power in the knee flexor muscles. Previous research reported the important role of knee flexor activation in jumping performance, particularly in the first phase of the countermovement (Nagano et al., 2005; Perraton et al., 2019).

No significant differences were found in the morphology of muscle bellies and tendons between the two groups and between participants with or without regeneration of the tendon. This is in contrast with previous studies reporting a larger cross-sectional area of the ST and GR regenerated tendons following ACL reconstruction (Gill et al., 2004; Snow et al.; 2012; Konrath et al., 2016). It may be argued that two months of neuromuscular electrical stimulations may not have been long enough to induce further morphological adaptations of muscles and tendons with respect to standard rehabilitation. The greater improvements in muscle strength and knee function in the NMES+ group than in the NAT group may therefore be ascribed mainly to neural adaptations of the trained muscles (Hortobágyi and Maffiuletti, 2011). The early NMES+ intervention might have acted against neural alterations that feature ACL rupture and surgery, such as arthrogenic muscle inhibition and atrophy of fast twitch motor units (Konishi et al., 2002). In addition, it cannot be excluded that the higher knee flexor strength in NMES+ participants might be due to an increase in strength of the SM and BF muscles, which are very close to the ST, and thus were involuntarily stimulated during training, i.e., the high intensity of stimulation and the size of the electrodes applied over the ST muscle might have also stimulated the adjacent SM and BF muscles. In contrast with a previous investigation (Konrath et al., 2016) reporting a compensatory increase in volume of the BF muscles in the operated limb of patients with harvested ST to counterbalance the loss of the ST muscle, in our study there were no between-limb differences in the volume of knee flexor muscles. However, Konrath et al. (2016) carried out the MRI for assessing muscle volume 2 years after surgery, whereas in this investigation it was performed at a mean of 12.5 months after surgery. Perhaps a longer-term assessment might have shown similar results.

Finally, there were no differences between the two groups in the number of patients showing tendon regeneration and none of the assessed parameters in this study were predictive of the regeneration of the tendon. The literature suggests that regeneration of the tendon may be linked to anatomical and surgical factors or to the outcome of the organization of precursor tendon cells in the post-surgical hematoma (Eriksson et al., 2003). To date, these latter factors appear to be the only variables affecting tendon regeneration. Future research should further clarify these points with studies assessing the effects of training interventions in larger cohorts of participants.

One last point needs to be discussed for this study. Only two groups of patients were included in the present study, i.e. the NMES+ group and a control group. We did not add a third group of patients performing only the additional functional exercises without superimposing NMES since in our previous research (Labanca et al. 2018) we found that adding only functional exercises without NMES to traditional rehabilitation does not have any effect on muscle strength in either the short or long-term following ACL surgery. In addition, a trend to an increase in knee joint pain was observed in patients performing only the additional exercises in comparison with those performing the additional exercises with the superimposition of the

NMES. In addition, in a more recent study (Borzuola et al. 2020), it was found that only superimposing NMES to voluntary muscle contractions leads to an increase of motoneuron excitability with respect to NMES only, without voluntary muscle contractions, or voluntary muscle contractions only, without NMES. Thus, it seems that in patients showing neural alteration and muscle inhibition, like in ALC reconstructed patients, the most effective way for an early recovery of muscle strength is functional exercise with superimposed NMES.

From a practical point of view, some methodological issues related to the intensity of stimulation during training should be discussed. It has been suggested that the intensity of stimulation should be increased as much as possible in accordance with the individual tolerance to maximize motor unit recruitment (Maffiuletti 2010). In this study, during quadriceps training three components of the same muscle were stimulated (VL, VM, and RF). The increase in the level of intensity is done according to the tolerance of the participants, meaning that the intensity is increased according to the tolerability of the "sense of effort" that the current creates in the muscle. During the training sessions the intensity of stimulation had to be adjusted for each of the three muscles as the same level of intensity gave different "sensations" in each muscle. In order to obtain the same "sense of effort" among the three muscles, the intensity of stimulation in the VM needed to be higher than in the VL and RF. In turn, the RF needed a lower level of intensity when compared with VL. One possible explanation is that VM shows a higher degree of atrophy, and inhibition, thus requiring a higher intensity to have the same "sense of effort" when compared with the other two muscles. Second, RF is continuously activated by non-functional strengthening exercises, such as straight leg raises, which are largely "used and abused" in the early phases after surgery. This kind of exercise is effective for muscles acting on the hip joint but has little effect on muscles acting directly and only on the knee joint as VM. In this light, the NMES+ exercise proposed in this study was highly effective for VM training, as shown by the results of the strength assessment 60 days after surgery, where the NMES+ group had higher levels of quadriceps strength at 30° in which VM has a higher role than the VL and RF. In addition, no differences were found between the two groups in the strength assessment at 90° where RF and VL plays the major role. To the best of the authors' knowledge, there are no studies within the literature reporting similar results in the early phase after surgery with other kinds of training interventions.

The main limitation of this study is that the gracilis muscle was not stimulated even if harvested for surgical graft. This choice was related to the fact that in a preliminary pilot investigation for this study, gracilis identification and stimulation was extremely difficult and resulted in high discomfort, making it impossible to appropriately increase the intensity of stimulation. A second limitation of the study is that the follow-up of participants ended at a mean of 12.5 months after surgery. In future studies follow up time should be extended. A third limitation of this study is that the sample size reached the minimum necessary to obtain a significant effect size based on an *a priori* calculation. Thus, results need to be confirmed by further studies involving a higher number of participants. In addition, the therapists who administered the NMES training were aware that patients were part of a research study. However, data collection and analysis were blinded since the authors performing the assessment and analysis were blinded on patients' allocation. Finally, it is well known that a unilateral ACL injury/surgery affects also contralateral limb strength and function (Wellsandt et al., 2017; Benjaminse et al., 2018). In this study, the additional NMES+ training was administered only in the operated limb, since this was a very early intervention aiming at an early reversal of hamstring muscle atrophy and inhibition in the operated limb. Longer term training interventions should also consider training both limbs.

Conclusion

In conclusion, the quality of rehabilitation can be greatly improved by adding a two-month structured resistance-training intervention, based on neuromuscular electrical stimulation superimposed on functional movements, in the early phase following ACL reconstruction with hamstring graft, as shown by the improvements in knee function and muscle strength in both the short and long term after surgery, regardless of tendon regeneration. Even if in the present study no significant NMES+ effects were observed, further studies are required to investigate whether extending this additional intervention beyond two months has an effect on tendon regeneration too.

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Key points

- For the first time, we have studied the effects of an innovative training intervention on muscle strength, morphology and knee function in a group of patients showing deficits due to degeneration of the semitendinosus muscle, which occurs following ACL reconstruction.
- Adding a two-month structured resistance-training intervention, based on neuromuscular electrical stimulation superimposed on functional movements, in the early phase following ACL reconstruction with hamstring graft, improves muscle strength and knee function both in the short and the long term.
- Patients undergoing the NMES- based strength training intervention show improvements in muscle strength and function regardless of tendon regeneration.

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