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Welling, Wouter; Benjaminse, Anne; Lemmink, Koen; DINGENEN, Bart & Gokeler, Alli (2019) Progressive strength training restores quadriceps and hamstring muscle strength within 7 months after ACL reconstruction in amateur male soccer players. In: Physical therapy in sport, 40, p. 10-18..

DOI: 10.1016/j.ptsp.2019.08.004
Handle: http://hdl.handle.net/1942/29146

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Reference (Published version):
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Progressive strength training restores quadriceps and hamstring muscle strength within 7 months after ACL reconstruction in amateur male soccer players

ABSTRACT

Objectives: The purpose of the current study was to compare the results of a progressive strength training protocol for soccer players after anterior cruciate ligament reconstruction (ACLR) with healthy controls, and to investigate the effects of the strength training protocol on peak quadriceps and hamstring muscle strength.

Design: Between subjects design.

Setting: Outpatient physical therapy facility.

Participants: Thirty-eight amateur male soccer players after ACLR were included. Thirty age-matched amateur male soccer players served as control group.

Main outcome measures: Quadriceps and hamstring muscle strength was measured at three time points during the rehabilitation. Limb symmetry index (LSI) >90% was used as cut-off criteria.

Results: Soccer players after ACLR had no significant differences in peak quadriceps and hamstring muscle strength in the injured leg at 7 months after ACLR compared to the dominant leg of the control group. Furthermore, 65.8% of soccer players after ACLR passed LSI >90% at 10 months for quadriceps muscle strength.

Conclusion: Amateur male soccer players after ACLR can achieve similar quadriceps and hamstring muscle strength at 7 months compared to healthy controls. These findings highlight the potential of progressive strength training in rehabilitation after ACLR that may mitigate commonly reported strength deficits.

Keywords: *anterior cruciate ligament; return to sport; isokinetic strength; strength training*

1. INTRODUCTION

One of the main components in early rehabilitation after anterior cruciate ligament reconstruction (ACLR) in soccer is restoring quadriceps and hamstring strength before on-field rehabilitation and return to sport (RTS) starts (Della Villa et al., 2012). Symmetrical quadriceps muscle strength prior to RTS has been suggested to be associated with a reduction in the re-injury risk (Kyritsis, Bahr, Landreau, Miladi, & Witvrouw, 2016; Grindem, Snyder-Mackler, Moksnes, Engebretsen, & Risberg, 2016). Furthermore, it has been reported that quadriceps muscle strength is associated with good self-reported knee function and patient satisfaction after ACLR (Logerstedt et al., 2014). It is common to calculate a limb symmetry index (LSI) for quadriceps and hamstring strength, defined as peak muscle strength of the injured leg divided by peak muscle strength of the non-injured leg x 100 (Lynch et al., 2015). To determine readiness for RTS, LSI criteria >90% are often used as cut-off scores (Lynch et al., 2015).

Unfortunately, recent studies showed that most patients after ACLR failed in passing RTS criteria for quadriceps muscle strength at 6 and 9 months after ACLR (Gokeler, Welling, Zaffagnini, Seil, & Padua, 2017; Toole, Ithurburn, Rauh, Hewett, Paterno, & Schmitt, 2017; Welling, Benjaminse, Seil, Lemmink, Zafagnini, & Gokeler, 2018). According to some researchers (Nagelli & Hewett, 2017), restoring quadriceps muscle strength requires prolonged rehabilitation after ACLR of up to a minimum of 2 years. Another perspective is to look critically at the content of rehabilitation. Muscle strength deficits following ACLR can be due to insufficient rehabilitation protocols (Thomee et al., 2011). Strength training intensity and volume might be too low to increase muscle strength and muscle volume to satisfactory levels (Gokeler et al., 2017; Welling et al., 2018). In addition, research emphasized the need for a more detailed documentation of strength training protocol after ACLR (Augustsson, 2013; Goff, Page, & Clark, 2018). The American College of Sports Medicine (ACSM) recommends that strength training must be completed with a frequency of two to three times per week, with two to four sets of exercises (8-12 repetitions) at 60%-80% (moderate to hard intensity) of one-repetition maximal (1RM) effort, including 2-3 min of rest between the exercises to regain muscle hypertrophy and strength in healthy individuals (Garber et al., 2011). By manipulating several aspects of the strength training (frequency, number of repetitions, unilateral and bilateral exercises), it is possible to perform strength training in a progressive manner (Garber et al., 2011; Ratamess et al., 2009; Schoenfeld, 2010). In addition, variation of exercises within strength training is suggested to enhance physical performance of the athlete (Ratamess et al., 2009; Schoenfeld, 2010).

Currently, most athletes after ACLR fail in passing RTS quadriceps muscle strength criteria and the ACSM has several recommendations for strength training to regain muscle strength. In addition, research found greater quadriceps deficits (lower LSI values) in patients after ACLR with a bone-patellar tendon-bone graft (BPTB) graft compared to a hamstring tendon graft (HT) using standardized rehabilitation (Welling et al., 2018). On the other hand, greater hamstring deficits were found in patients after ACLR with HT graft compared to BPTB graft (Hughes et al., 2019).

The primary purpose of the current study was to compare the results of a strength training protocol for soccer players after ACLR with healthy controls, and to investigate the effects of the strength training protocol on peak quadriceps and hamstring muscle strength and self-reported knee function during rehabilitation after ACLR. The secondary purpose was to investigate the differences between soccer players after ACLR with HT graft and BPTB graft in peak quadriceps and hamstring muscle strength during the course of rehabilitation after ACLR. It was hypothesized that soccer players after ACLR showed comparable peak quadriceps and hamstring muscle strength and LSI values after training compared to healthy controls. Additionally, it was hypothesized that peak quadriceps and hamstring muscle strength significantly improves over time as well as self-reported knee function as a result of the strength training. Also, it was hypothesized that soccer players after ACLR with HT graft show greater peak quadriceps muscle strength and weaker peak hamstring muscle strength compared to those with a BPTB graft.

2. MATERIALS AND METHODS

2.1 Participants

Thirty-eight amateur male soccer players (age 24.2±4.7 years) after ACLR participated in this study. The soccer players were recruited one-to-one in person in the physical therapy facility based on the inclusion criteria. For 29 soccer players after ACLR (76.3%) the injured leg was the dominant leg, defined as the preferred leg to kick a ball (Padua, Marshall, Boling, Thigpen, Garrett, & Beutler, 2009; Welling, Benjaminse, Gokeler, & Otten, 2016). A power analysis (G*Power, Version 3.1.7) was used to calculate the required sample size for the soccer players after ACLR. With an effect size of 0.50 (medium effect ANOVA) and an alpha of 0.05, 34 patients after ACLR were required to obtain a power of 0.80 based on peak quadriceps and hamstring muscle strength as outcome measure (Cohen, 1988).

Inclusion criteria for the soccer players after ACLR were: 1) age between 18 and 35 years old, 2) participating in competitive soccer (amateur level in the Netherlands) playing at least four hours a week (training and match), 3) primary isolated ACL lesion and 4) arthroscopic ACLR (HT graft or BPTB graft, based on the preference of the orthopaedic surgeon) with an anteromedial portal technique. All soccer players after ACLR underwent a rehabilitation protocol, including strength training based on ACSM guidelines (Garber et al., 2011) at the same outpatient physical therapy facility. Exclusion criteria were: 1) presence of pain and/or swelling (Visual Analogue Scale ≤ 3) of the injured knee during a test moment (effusion measured with the sweep test, grade ≥ 0) (Sturgill, Snyder-Mackler, Manal, & Axe, 2009), 2) no ambition to return to competitive soccer 3) a feeling of instability in the injured knee or 4) meniscal and/or cartilaginous lesions ≥ grade 3 (Grindem et al., 2016). Before data collection, all soccer players after ACLR signed an informed consent. The study was approved by the Review Board at the University of XXX. Data collection took place between August 2016 and March 2018 in the same outpatient physical therapy clinic.

Thirty male age-matched amateur soccer players (age 22.8±2.5 years) served as a control group (fourth division amateur level in the Netherlands). Inclusion criteria were as follows: 1) age between 18 and 35 years old, 2) no history of knee injuries at all, 3) physically active in competitive soccer for a minimum of four hours per week. The control group was tested once, at a rest day in a regular training week. Descriptive data of both groups are presented in Table 1. Soccer players in the control group signed an informed consent before data collection.

2.2 Strength training protocol

The strength training protocol was based on the ACSM principles and rehabilitation guidelines from earlier studies (Gokeler et al., 2017; Welling et al., 2018; Myer, Paterno, Ford, Quatman, & Hewett, 2006; Myer, Paterno, Ford, & Hewett, 2008). The strength training protocol was divided in four phases (Figure 1) and soccer players after ACLR had to meet criteria before entering the next phase of the rehabilitation (Myer et al., 2006; Myer et al., 2008; Rambaud, Ardern, Thoreux, Regnaux, & Edouard, 2018; Karasel et al., 2010). The first two weeks, soccer players after ACLR were advised to do leg raises at home, walk with crutches and rest for wound healing. The initial phase started two weeks after the ACLR and focused on attaining full knee extension, reduction of pain and quadriceps activation exercises (Gokeler, Bisschop, Benjaminse, Myer, Eppinga & Otten, 2014; Myer et al., 2006; Myer et al., 2008). Every session of the strength training was performed under supervision of a physiotherapist. In addition, soccer players after ACLR had to meet specific strength criteria for returning to different activities (Table 2). The soccer players after ACLR trained in the physical therapy clinic with a mean frequency of 2.6±0.7 times per week. The second phase started with relatively easy to perform muscular endurance exercises using maximal 2 sets of 15-25 repetitions (intensity <50% of 1RM including 2-3 minute rest between sets) (Garber et al., 2011), such as a step-up exercises, leg raise exercises or leg press exercises. Open kinetic chain exercises with resistance were not performed until the third phase of the strength training protocol. Based on the 24-hour reaction of the knee (no increase in joint effusion or pain and presence of minimal pain on the Visual Analogue Scale of ≤3/10 after a physical therapy session reported by the athlete), exercises were added or the intensity of the exercises was progressed. The general duration of the second phase was 10 to 14 weeks. At the end of the second phase, the first test session was conducted.

The goal of the third phase of the ACLR rehabilitation was to improve strength and normalize leg strength symmetry (based on the first isokinetic strength test at 4 months) (Myer et al., 2006). In addition to muscular strength and endurance training, other exercises such as balance exercises, running and jump-landing technique were trained during the third phase of the rehabilitation. Muscle endurance was trained using maximal two sets of 15-25 repetitions (intensity <50% of 1RM), including 2-3 minute rest (Garber et al., 2011). Lower extremity strength training consisted of both one-legged and two-legged exercises. From the third phase forward both closed and open kinetic chain exercises under resistance were performed. Common open kinetic chain exercises were leg extension and leg curl. The leg extension was performed with a range of motion of 90°-45° (Figure 2). The range of motion was progressed during the rehabilitation to full range of motion. Common closed kinetic chain exercises were squats, deadlifts, split squats, step-ups and good mornings (Figure 3). To offer variation in the strength training for the athlete, alternative exercises of the aforementioned exercises were also used, such as back squats, front squats, sumo squats and pistol squats (Figure 3). Joint angles during these exercises did not exceed 90° knee flexion. During a training session, unilateral and bilateral strength exercises were combined and performed at 2-4 sets of 8-10 repetitions (intensity 60%-80% of 1RM), all with 2-3 minutes of rests between sets. Furthermore, a pyramid training form was performed including four sets of 14-12-10-8 repetitions, all with 2-3 minutes rests between sets. The third phase had a general duration of 12 to 14 weeks. At the end of the third phase, the second test session was conducted.

The goal of the fourth phase was to address the remaining knee extension and flexion muscular strength deficits. Based on the results of the second isokinetic strength test (at 7 months), the strength training protocol was tailored to address these strength symmetry deficiencies. For maximal strength and hypertrophy the exercises could be progressed further to 5 sets of 3 repetitions (intensity >80% of 1RM) including 2-3 minutes of rest to improve maximal muscular strength (Garber et al., 2011). Physiological responses of the knee joint (for example pain, swelling and oedema after training) were constantly evaluated and if necessary, training was adjusted based on these responses. In addition, muscle endurance was trained using maximal two sets of

15-25 repetitions (intensity <50% of 1RM), including 2-3 minute rest (Garber et al., 2011). Additionally, soccer players after ACLR were specifically instructed to perform the concentric part of the exercise in an explosive manner (“as fast as possible”). For eccentric exercises as leg press and Nordic hamstring curl, soccer players after ACLR were instructed to perform the eccentric part of the exercise “as slow as possible” (5-6 seconds). In the fourth phase of the ACLR rehabilitation, the same exercises and possible variations were used as in the previous stages. After the last isokinetic strength test (at 10 months after ACLR, at the moment of RTS) any strength deficits were addressed by tailoring the muscular strength and endurance training protocol based on these deficits. The general duration of the fourth phase was 14 to 16 weeks. Besides strength training, other aspects (i.e., balance, running technique, jump-landing technique, etc.) were trained during the fourth phase of the rehabilitation. After that, the focus was on on-field rehabilitation and RTS (Myer et al., 2006; Myer et al., 2008, Buckthorpe, 2019).

2.3 Strength measurements

Peak quadriceps and hamstring muscle strength was measured at three different moments during the rehabilitation: at 4 months, at 7 months and at 10 months after ACLR. During the last isokinetic strength test, all soccer players after ACLR were in the final phase of their rehabilitation, before RTS (Buckthorpe, 2019). Some soccer players after ACLR could not do the strength test at the ideal moment in their rehabilitation (4, 7 and 10 months after ACLR) due the presence of swelling and/or pain at the moment of testing. Therefore, there was some variation in the time points of testing. Body weight was measured before the first test session. Before testing, the soccer players after ACLR and controls performed a 10-minute warm-up on a stationary bike at low intensity. Concentric peak muscle strength of both legs was tested with an isokinetic device (Biodex System 3; Biodex Medical Systems, Inc, Shirley, NY), which has been shown to be highly reliable (test-retest reliability ICC 0.91-0.99) (Tiffreau, Ledoux, Eymard, Thevenon, & Hogrel, 2007), with a minimal detectable change (MDC) of isokinetic peak quadriceps muscle strength of 33.9 Nm at a velocity of 60°/s (Kean, Birmingham, Garland, Bryant, & Giffin, 2010). The soccer players after ACLR and controls were seated in an upright position and fixed to the testing apparatus, with the straps around the pelvis, the thigh and malleoli. The range of motion was set as 100° flexion to 0° extension. The axis of rotation of the dynamometer was aligned with the lateral femoral epicondyle. An average of three submaximal repetitions was performed to familiarize the soccer players after ACLR and controls with the test protocol. Five maximal concentric repetitions for flexion and extension were conducted at a velocity of 60°/s (Figure 4), as recommended (Undheim et al., 2015). The non-injured leg was always tested first with a rest period of 1 minute between legs. For the control group, the non-dominant leg was always tested first. Standard verbal encouragement was given during each test. After each of the three strength tests, soccer players after ACLR completed the International Knee Documentation Committee Subjective Knee Form (IKDC) questionnaire for self-reported knee function (Irrgang et al., 2001).

2.4 Data reduction

Isokinetic data was exported to SPSS version 20 (IBM SPSS Inc, Chicaco, IL). Three dependent variables were analyzed; peak torque muscle strength (Nm), peak torque quadriceps muscle strength normalized to bodyweight (PT/BW, Nm/kg) (Harbo, Brincks, & Andersen, 2012; Lue, Chang, Chen, Lin, & Chen, 2000) and LSI values. PT/BW values were calculated by dividing the quadriceps peak torque at 60°/s with BW. A threshold for isokinetic quadriceps muscle strength at 60°/s after ACLR has been recommended as >3.0 Nm/kg (Kuenze, Hertel, Saliba, Diduch, Weltman, & Hart, 2015). LSI values were calculated for peak quadriceps and hamstring muscle strength by dividing the injured leg with the non-injured leg x 100 (Lynch et al., 2015). For the control group, LSI values were calculated for peak quadriceps and hamstring muscle strength by dividing the weakest leg by the strongest leg (dominant leg or non-dominant leg) x 100 because of the fact that the dominant leg was not always the strongest leg in the control group.

2.5 Statistical analysis

Data normality was analyzed with the Shapiro-Wilk test (Ghasemi & Zahediasl, 2012). All data were normally distributed as analyzed with SPSS version 20 (IBM SPSS 244 Inc, Chicago, IL). To determine differences in peak quadriceps and hamstring muscle strength and LSI values across time (4 months, 7 months and 10 months), between legs (non-injured leg and the injured leg) and groups (ACLR and controls), a 3x2x2 ANOVA were conducted. Additionally, the percentages of soccer players after ACLR and controls passing the LSI >90% (Lynch et al., 2015) and >3.0 Nm/kg (Kuenze et al., 2015) were calculated. Also, IKDC values of the soccer players after ACLR were compared with normative IKDC values from previous research (males; 89.7–85.1, females; 83.9–82.8) (Logerstedt et al., 2014; Gokeler et al., 2017). An additional ANOVA was conducted to determine difference in peak quadriceps and hamstring muscle strength and LSI values between soccer players with an ACLR with HT graft and soccer players with an ACLR with BPTB graft.

3. RESULTS

3.1 Main findings

Analysis of the demographic variables between groups showed that the soccer players after ACLR had more body weight compared to the control group (79.0±13.3 vs. 72.7±6.8 kg; p=0.018) (Table 1). The soccer players after ACLR had significant weaker peak quadriceps muscle strength in the injured leg at 4 months compared to the dominant leg of the control group (188.6±51.6 vs. 231.7±27.0 Nm; p<0.001) (Table 3). At 7 months however, there were no significant differences in peak quadriceps muscle strength or peak hamstring muscle strength in the injured leg compared to the dominant leg of the control group (peak quadriceps muscle strength: 223.4±51.1 vs. 231.7±27.0 Nm; p=0.052, peak hamstring muscle strength: 143.8±29.9 vs. 136.3±21.1 Nm; p=0.250). At 10 months, the soccer players after ACLR had greater peak hamstrings muscle strength in the injured leg compared to the dominant leg of the control group (149.5±31.2 vs. 136.3±21.1 Nm; p=0.007).

For PT/BW values, for the first two time points the soccer players after ACLR had significant lower values for quadriceps muscle strength in the injured leg (4 months 2.4±0.5 vs. 3.2±0.3 Nm/kg; p<0.001; 7 months 2.9±0.5 vs. 3.2±0.3 Nm/kg; p=0.007) compared to the dominant leg of the control group. This difference was no longer present at 10 months after ACLR.

The LSI values for the soccer players after ACLR for both quadriceps and hamstring muscle strength significantly increased over time (Table 3, Figure 5). In addition, PT/BW values for quadriceps muscle strength significantly increased over time (Table 4). At 10 months, 65.8% of the soccer players after ACLR passed LSI >90% for quadriceps muscle strength and 76.3% for hamstring muscle strength. Also, 71.1% of the soccer players after ACLR passed PT/BW >3.0 Nm/kg for quadriceps muscle strength at 10 months (Table 4).

3.2 Self-reported knee function

The mean IKDC score of the soccer players after ACLR was significantly higher at 7 months compared to 4 months (78.0±8.6 vs. 68.0±6.0; p<0.001) and significantly higher at 10 months compared to 4 months (86.5±5.4 vs. 78.0±8.6; p<0.001).

3.3 Between graft comparisons

An ACLR with BPTB graft showed greater peak hamstring muscle strength in the injured leg at 4 months (149.9±22.5 vs. 127.2±26.9 Nm; p=0.007), at 7 months (156.5±23.6 vs. 136.3±25.8 Nm; p=0.010) and at 10 months (160.6.6±30.1 vs. 139.3±25.7 Nm; p=0.010) compared to an ACLR with HT graft. No significant differences were found in peak quadriceps muscle strength between an ACLR with BPTB graft and an ACLR with HT graft for all time points. A significant higher LSI value was found for quadriceps muscle strength in an ACLR with HT graft compared to an ACLR with BPTB graft at 7 months (90.3±12.4 % for an ACLR with HT graft vs. 75.1±12.2 % for an ACLR with BPTB graft; p=0.001) and at 10 months (98.3±8.4 % for an ACLR with HT graft vs. 87.1±12.5 % for an ACLR with BPTB graft; p=0.002).

4. DISCUSSION

4.1 Main findings

The primary findings of the current study were that soccer players 7 months after ACLR showed no significant differences in peak quadriceps and hamstring muscle strength compared to the control group. At 10 months, the soccer players after ACLR were stronger than control group. Furthermore, 65.8% of the soccer players after ACLR passed LSI >90% at 10 months for quadriceps muscle strength and 76.3% for hamstring muscle strength. Additionally, self-reported knee function progressed over time. The secondary finding showed that soccer players with an ACLR with BPTB showed greater peak hamstring muscle strength at 4 months, 7 months and 10 months compared to soccer players with an ACLR with HT. Furthermore, higher LSI values for quadriceps muscle strength in soccer players with an ACLR with HT graft were found at 7 and 10 months compared to an ACLR with a BPTB graft.

The absolute increase in quadriceps muscle strength for the soccer players after ACLR was 33.3-34.8 Nm per three months time increment, which is similar to the MDC of 33.9 Nm for quadriceps muscle strength (Kean et al., 2010). These findings indicate a clinical important improvement in quadriceps muscle strength from 4 to 7 and from 7 to 10 months after ACLR. Symmetrical quadriceps muscle strength is suggested to be essential in safe RTS as it decreases the re-injury rate significantly (Grindem et al., 2016). Asymmetrical quadriceps muscle strength is associated with altered knee biomechanics during functional tests, which has been found as risk factors for an ACL re-injury (Palmieri-Smith & Lepley, 2015). In addition, quadriceps weakness is suggested to be a risk factor for developing knee osteoarthritis (Palmieri-Smith & Lepley, 2015). Earlier studies of our research group showed that athletes after ACLR failed in passing RTS criteria for quadriceps muscle strength both at 6 months and 9 months after ACLR (Gokeler et al., 2017; Welling et al., 2018). The current study is part of an ongoing project and we have reviewed the ACLR rehabilitation protocol critically and changed the rehabilitation protocol by including ACSM principles of strength training and principles of earlier studies (Gokeler et al., 2017; Welling et al., 2018; Myer et al., 2006; Myer et al., 2008). As a result, the soccer players after ACLR in the current study showed comparable quadriceps muscle strength to controls and more symmetrical quadriceps muscle strength compared to earlier results with a standardized rehabilitation protocol (Gokeler et al., 2017; Toole et al., 2017; Welling et al., 2018). Absolute peak quadriceps and hamstring muscle strength values at 10 months in the injured leg are greater compared to earlier published work of our research group around 9 months after ACLR (peak quadriceps muscle strength 256.7±51.0 Nm in the current study vs. 223.9±44.4 Nm in an earlier study; peak hamstring muscle strength 149.5±31.2 Nm in the current study vs. 134.1±32.1 Nm in an earlier study) (Welling et al., 2018). In addition, increased PT/BW values for quadriceps muscle strength in the injured leg were achieved (3.2±0.6 Nm/kg in the current study vs. 3.0±0.6 Nm/kg in an earlier study) (Welling et al., 2018). These findings indicate that the strength training protocol used in the current study result in greater quadriceps and hamstring muscle strength in contrast to the standardized rehabilitation protocol used in earlier studies. In the current study, we started hypertrophy training and open kinetic chain exercises under resistance in the third phase of the strength training protocol. However, recent research suggests that open chain exercises are beneficial for regaining quadriceps muscle strength and therefore, should be included earlier (from 4 weeks postoperative for an ACLR with HT) in the ACLR rehabilitation in a restricted range of motion 90°-45° (van Melick et al., 2016; Perriman, Leahy, & Semciw, 2018). Future research should investigate the effects of earlier included hypertrophy training and open kinetic chain exercises.

At the last time point, the percentage of soccer players after ACLR passing LSI >90% for quadriceps muscle strength (65.8%) is higher compared to others. Toole et al. reported that 43.5% passed the LSI >90% around 8 months after ACLR (Toole et al., 2017). In Welling et al., 53.2% passed the LSI >90% around 9 months after ACLR (Welling et al., 2018). These findings indicate that the strength training protocol used in the current study may be more effective in contrast to the traditional standardized rehabilitation protocol. However, caution is warranted when using only LSI values in the RTS decision making since LSI values can potentially mask bilateral deficits and therefore, overestimate performance (Gokeler, Welling, Benjaminse, Lemmink, Seil, & Zaffagnini, 2017). Therefore, it is suggested to use a PT/BW value which is thought to be a more adequate method when analyzing strength data (Dingenen & Gokeler, 2017; Welling et al., 2018). At the second time point (7 months after ACLR), 61.5% of the soccer players after ACLR passed the >3.0 Nm/kg threshold for quadriceps muscle strength for the injured leg and 71.1% at the last time point (10 months after ACLR). These results are higher in contrast to our earlier results (27.4% at 6 months for the injured leg and 40.3% at 9 months (Welling et al., 2018), suggesting that the previously used rehabilitation protocol after ACLR might be not sufficient enough. The results of the current study show that 65.8% of the soccer players after ACLR can pass RTS quadriceps muscle strength criteria 10 months after ACLR. This can be reached as long as soccer players after ACLR train consistently (mean frequency 2.6 sessions per week) and with the appropriate training volume and intensity. Research suggests that the motivation and adherence during the rehabilitation after ACLR are essential, since most athletes after ACLR fail to achieve RTS quadriceps muscle strength criteria at 6 and 9 months after ACLR (Gokeler et al., 2017; Welling et al., 2018). Therefore, it is advised that clinicians should include variation of exercises, create challenges and employ sport specific training within the rehabilitation to keep the motivation and adherence high (Chan, Lonsdale, Ho et al., 2009). Additionally, it needs to be mentioned here though that altered loading of the injured leg may be a cause and/or effect of quadriceps weakness (Hart, Ko, Konold, & Pietrosimone, 2010; Sigward, Chan, Lin, Almansouri, & Pratt, 2019). If soccer players after ACLR continue to avoid physiological loading of the injured leg, quadriceps muscle strength may not be restored at all, no matter how hard they train (Gokeler, Bisschop, Benjaminse, Myer, Eppinga, & Otten, 2014). Also, insight gained from motor learning research may improve the effectiveness in developing muscle strength during rehabilitation (Gokeler et al., 2013). More functional neuromuscular training methods should be added to strengthening training to effectively targeting asymmetrical movement patterns in soccer players after ALCR (Benjaminse, Holden, & Myer, 2018; Buckthorpe, La Rosa, & Della Villa, 2019).

Traditionally, RTS was recommended 6 months after ACLR (Barber-Westin & Noyes, 2011). However, the results of the current study showed improvement in peak quadriceps and hamstring muscle strength between 7 and 10 months, indicating that extending the rehabilitation until around 10 months results in greater quadriceps and hamstring muscle strength. Therefore, it is advised to extend the rehabilitation until at least 10 months after ACLR, also because of the persistence of strength deficits which may be present until 2 years after ACLR (Nagelli & Hewett, 2017). In addition, despite the consistent and intensive strength training still 34.2% of the soccer players after ACLR failed the LSI >90% criteria for quadriceps muscle strength and 28.9% failed the >3.0 Nm/kg threshold for the injured leg at 10 months. The decision for RTS after ACLR should be a criteria and time based combination. Therefore, it is advised to extend the rehabilitation and train more frequent with a higher intensity until strength criteria, among other criteria, are passed.

4.2 Self-reported knee function

Recent research showed a lack of clinical improvement in IKDC score during standardized rehabilitation after ACLR (Welling et al., 2018). The soccer players after ACLR in the current study had an average IKDC score of 68.0±6.0 at 4 months, 78.0±8.6 at 7 months and 86.5±5.4 at 10 months. At the first two time points (4 months and 7 months after ACLR), the majority of soccer players after ACLR scored below the cut-off scores (males; 89.7–85.1, females; 83.9–82.8) (Logerstedt et al., 2014; Gokeler et al., 2017), which indicates lower self-reported knee function 4 and 7 months after ACLR compared to healthy controls. The absolute change in IKDC score was 8.5-10.0 per three months time increment, which is similar to the MDC of 8.8 and therefore indicate clinical important improvements (Grevnerts, Terwee, & Kvist, 2015). At 10 months, soccer players after ACLR reached the cut-off scores (Logerstedt et al., 2014), which indicate good self-reported knee function. The combination of both greater peak quadriceps and hamstring muscle strength and better self-reported knee function, shows great potential of the inclusion of progressive strength training during rehabilitation.

4.3 Between graft comparisons

Between graft comparison showed that soccer players with an ACLR with BPTB graft had greater absolute peak hamstring muscle strength in the injured leg at all three time points (4, 7 and 10 months after ACLR) compared to soccer players with an ACLR with

HT graft. These findings are in line with earlier research showing more hamstring weakness in athletes with an ACLR with HT (Hughes et al., 2019). In addition, soccer players with an with HT graft showed a higher LSI value for quadriceps muscle strength in contrast to soccer players with an ACLR with BPTB graft at 7 and 10 months. More in detail, at 4 months 12.5% of the soccer players with an ACLR with HT graft passed the LSI >90% for quadriceps muscle strength and 50.0% at 7 months, compared to no soccer players with an ACLR with BPTB graft at both 4 and 7 months. Furthermore, at 10 months 83.3% of the soccer players with an ACLR with HT graft passed the LSI >90% for quadriceps muscle strength in contrast to only 35.7% of the soccer players with an ACLR with BPTB graft. These findings are in line with previous research, showing a greater quadriceps deficit in athletes with an ACLR with BPTB graft compared to an ACLR with HT graft (Welling et al., 2018; Machado, Debieux, Kaleka, Astur, Peccin, & Cohen, 2018). Is it suggested that rehabilitation after ACLR should be tailored based on the graft type and future research should focus on more specific rehabilitation for both ACLR's with HT and BPTB graft.

4.4 Study limitations

There are some limitations that should be noticed. The current study focused on amateur male soccer players after ACLR and therefore, the results can not be generalized to other gender, type and level of sports. Secondly, the results of the current study could be influenced by crossover effects of other aspects besides strength training (for example balance training, jumping- or running exercises) within the rehabilitation of the ACLR. Third, the number of the supervised sessions in the current study could not be generalized to other countries since the health insurance systems differ between countries.

5. CONCLUSIONS

The results show that by using principles of progressive strength training, soccer players who underwent an ACLR regain quadriceps and hamstring muscle strength comparable to healthy controls at 7 months after ACLR. At 10 months, the soccer players after

ACLR were stronger compared to healthy controls. In addition, passing LSI >90% for quadriceps muscle strength was achieved by 65.8% of the soccer players after ACLR and 76.3% for hamstrings strength 10 months after ACLR. Also, soccer players after ACLR showed good self-reported knee function 10 months after ACLR. These findings highlight the potential of progressive strength training in rehabilitation after ACLR that may mitigate commonly reported strength deficits. Physiotherapists should focus on improving the quality of the rehabilitation after ACLR, by implementing more progressive strength training.

REFERENCES

Augustsson J. Documentation of strength training for research purposes after ACL reconstruction. Knee Surg Sports Traumatol Arthrosc. 2013;21(8):1849-1855.

Cohen J, Statistical power analysis for the behavioral sciences. Lawrence Erlbaum Associate, Hillsdale. 1988.

Barber-Westin SD, Noyes FR. Factors used to determine return to unrestricted sports activities after anterior cruciate ligament reconstruction. Arthroscopy. 2011;27(12):1697–705.

Benjaminse A, Holden S, Myer GD. ACL rupture is a single leg injury but a double leg problem: too much focus on 'symmetry' alone and that's not enough! Br J Sports Med. 2018;52(16):1029-1030.

Buckthorpe M. Optimising the late-stage rehabilitation and return-to-sport training and testing process after ACL reconstruction. Sports Med. 2019;49(7):1043-1058.

Buckthorpe M, La Rosa G, Villa FD. Restoring knee extensor strength after anterior cruciate ligament reconstruction: a clinical commentary. Int J Sports Phys Ther. 2019;14(1):159-172.

Chan DK, Lonsdale C, Ho PY, et al. Patient motivation and adherence to postsurgery rehabilitation exercise recommendations: the influence of physiotherapists' autonomy-supportive behaviors. Arch Phys Med Rehabil. 2009;90(12):1977–82.

Della Villa S, Boldrini L, Ricci M, Danelon F, Snyder-Mackler L, Nanni G, et al. Clinical outcomes and return-to-sports participation of 50 soccer players after anterior cruciate ligament reconstruction through a sport-specific rehabilitation protocol. Sports Health. 2012;4(1):17-24.

Dingenen B, Gokeler A. Optimization of the return-to-sport paradigm after anterior cruciate ligament reconstruction: a critical step back to move rorward. Sports Med. 2017.

Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc. 2011;43(7):1334-1359.

Ghasemi A, Zahediasl S. Normality tests for statistical analysis: a guide for non-statisticians. Int J Endocrinol Metab. 2012 Spring; 10(2): 486–489.

Grindem H, Snyder-Mackler L, Moksnes H, Engebretsen L, Risberg MA. Simple decision rules can reduce reinjury risk by 84% after ACL reconstruction: the Delaware-Oslo ACL cohort study. Br J Sports Med. 2016. doi:10.1136/bjsports-2016-096031.

Goff AJ, Page WS, Clark NC. Reporting of acute programme variables and exercises descriptors in rehabilitation strength training for tibiofemoral joint soft tissue injury: a systematic review. Phys Ther Sport. 2018;34:227-237. doi: 10.1016/j.ptsp.2018.10.01

Gokeler A, Benjaminse A, Hewett TE, Paterno MV, Ford KR, Otten E, et al. Feedback techniques to target functional deficits following anterior cruciate ligament reconstruction: implications for motor control and reduction of second injury risk. Sports Med. 2013;43(11):1065-1074.

Gokeler A, Bisschop M, Benjaminse A, Myer GD, Eppinga P, Otten E. Quadriceps function following ACL reconstruction and rehabilitation: implications for optimisation of current practices. Knee Surg Sports Traumatol Arthrosc. 2014;22(5):1163-74.

Gokeler A, Welling W, Benjaminse A, Lemmink K, Seil R, Zaffagnini S. A critical analysis of limb symmetry indices of hop tests in athletes after anterior cruciate ligament reconstruction: A case control study. Orthop Traumatol Surg Res. 2017;103(6):947–951.

Gokeler A, Welling W, Zaffagnini S, Seil R, Padua D. Development of a test battery to enhance safe return to sports after anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc. 2017;25(1):192-199.

Grevnerts HT, Terwee CB, Kvist J. The measurement properties of the IKDC-subjective knee form. Knee Surg Sports Traumatol Arthrosc. 2015;23(12):3698–3706.

Harbo T, Brincks J, Andersen H. Maximal isokinetic and isometric muscle strength of major muscle groups related to age, body mass, height, and sex in 178 healthy subjects. Eur J Appl Physiol. 2012;112(1):267-275.

Hart JM, Ko JW, Konold T, Pietrosimone B. Sagittal plane knee joint moments following anterior cruciate ligament injury and reconstruction: a systematic review. Clin Biomech. 2010;25(4):277–283.

Hughes JD, Burnham JM, Hirsh A, Musahl V, Fu FH, Irrgang JJ, et al. Comparison of Short-term Biodex Results After Anatomic Anterior Cruciate Ligament Reconstruction Among 3 Autografts. Orthop J Sports Med 2019;31;7(5):2325967119847630.

Irrgang JJ, Anderson AF, Boland AL, Harner CD, Kurosaka M, Neyret P, Richmond JC, Shelborne KD. Development and validation of the international knee documentation committee subjective knee form. AM J Sports Med. 2001;29(5):600-13.

Karasel S, Akpinar B, Gulbahar S, Baydar M, El O, Pinar H, et al. Clinical and functional outcomes and proprioception after a modified accelerated rehabilitation program following anterior cruciate ligament reconstruction with patellar tendon autograft. Acta Orthop Traumatol Turc. 2010;44(3):220-228.

Kean CO, Birmingham TB, Garland SJ, Bryant DM, Giffin JR. Minimal detectable change in quadriceps strength and voluntary muscle activation in patients with knee osteoarthritis. Arch Phys Med Rehabil. 2010;91(9):1447-1451.

Kuenze C, Hertel J, Saliba S, Diduch DR, Weltman A, Hart JM. Clinical thresholds for quadriceps assessment after anterior cruciate ligament reconstruction. J Sport Rehab. 2015;24(1):36–46.

Kyritsis P, Bahr R, Landreau P, Miladi R, Witvrouw E. Likelihood of ACL graft rupture: not meeting six clinical discharge criteria before return to sport is associated with a four times greater risk of rupture. Br J Sports Med. 2016;50: 946–951.

Logerstedt D, Di Stasi S, Grindem H, Lynch A, Eitzen I, Engebretsen L, Risberg MA, Axe MJ, Snyder-Mackler L. Selfreported knee function can identify athletes who fail return to activity criteria up to 1 year after anterior cruciate ligament reconstruction: a Delaware-Oslo ACL cohort study. J Orthop Sports Phys Ther. 2014;44(12):914–923.

Lue YJ, Chang JJ, Chen HM, Lin RF, Chen SS. Knee isokinetic strength and body fat analysis in university students. Kaohsiung J Med Sci. 2000;16(10):517-524.

Lynch AD, Logerstedt DS, Grindem H, Eitzen I, Hicks GE, Axe MJ, Engebretsen L, Risberg MA, Snyder-Mackler L. Consensus criteria for defining 'successful outcome' after ACL injury and reconstruction: a Delaware-Oslo ACL cohort investigation. Br J Sports Med. 2015;49(5):335–342.

Machado F, Debieux P, Kaleka CC, Astur D, Peccin MS, Cohen M. Knee isokinetic performance following anterior cruciate ligament reconstruction: patellar tendon versus hamstrings graft. Phys Sportsmed. 2018;46(1):30-35.

Myer GD, Paterno MV, Ford KR, Hewett TE. Neuromuscular training techniques to target deficits before return to sport after anterior cruciate ligament reconstruction. J Strength Cond Res. 2008;22(3):987-1014.

Myer GD, Paterno MV, Ford KR, Quatman CE, Hewett TE. Rehabilitation after anterior cruciate ligament reconstruction: criteria-based progression through the return-to-sport phase. J Orthop Sports Phys Ther. 2006;36(6):385-402.

Nagelli CV, Hewett TE. Should return to sport be delayed until 2 years after anterior cruciate ligament reconstruction? Biological and functional considerations. Sports Med. 2017;47(2):221-232.

Padua DA, Marshall SW, Boling MC, Thigpen CA, Garrett WE Jr, Beutler AI. The Landing Error Scoring System (LESS) is a valid and reliable clinical assessment tool of jump landing biomechanics: the JUMP-ACL study. Am J Sports Med. 2009;37(10):1996–2002.

Palmieri-Smith RM, Lepley LK. Quadriceps strength asymmetry after anterior cruciate ligament reconstruction alters knee joint biomechanics and functional performance at time of return to activity. Am J Sports Med. 2015;43(7):1662-1669.

Perriman A, Leahy E, Semciw AI. The effect of open- versus closed-kinetic-chain exercises on anterior tibial laxity, strength, and function following anterior cruciate ligament reconstruction: a systematic review and meta-analysis. J Orthop Sports Phys Ther. 2018;48(7):552-566.

Rambaud AJM, Ardern CL, Thoreux P, Regnaux JP, Edouard P. Criteria for return to running after anterior cruciate ligament reconstruction: a scoping review. Br J Sports Med. 2018;52(22):1437-1444.

Ratamess NA, Alvar BA, Evetoch TK, Housh TJ, Kibler B, Kraemer WJ, Triplett NT. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. Med Sci Sports Exerc. 2009;41:687–708.

Schoenfeld BJ. The mechanisms of muscle hypertrophy and their application to resistance training. J Strength Cond Res. 2010;24:2857–2872.

Sigward SM, Chan MM, Lin PE, Almansouri SY, Pratt KA. Compensatory strategies that reduce knee extensor demand during a bilateral squat change from 3 to 5 months following anterior cruciate ligament reconstruction. J Orthop Sports Phys Ther. 2018;48(9):713-718.

Tiffreau V, Ledoux I, Eymard B, Thevenon A, Hogrel JY. Isokinetic muscle testing for weak patients suffering from neuromuscular disorders: a reliability study. Neuromuscul Disord. 2007;17(7):524–531.

Thomee R, Kaplan Y, Kvist J, Myklebust G, Risberg MA, Theisen D, et al. Muscle strength and hop performance criteria prior to return to sports after ACL reconstruction. Knee Surg Sports Traumatol Arthrosc 2011;19(11):1798-1805.

Toole AR, Ithurburn MP, Rauh MJ, Hewett TE, Paterno MV, Schmitt LC. Young athletes after anterior cruciate ligament reconstruction cleared for sports participation: how many actually meet recommended return-to-sport criteria cutoffs? J Orthop Sports Phys Ther. 2017;07:1-27.

Undheim MB, Cosgrave C, King E, Strike S, Marshall B, Falvey E, et al. Isokinetic muscle strength and readiness to return to sport following anterior cruciate ligament reconstruction: is there an association? A systematic review and a protocol recommendation. Br J Sports Med. 2015;49(20):1305-1310.

Van Melick N, van Cingel REH, Brooijmans F et al. Evidence- based clinical practice update: practice guidelines for anterior cruciate ligament rehabilitation based on systematic review and multidisciplinary consensus. Br J Sports Med. 2016;50:1506–1515.

Welling W, Benjaminse A, Gokeler A, Otten B. Enhanced retention of drop vertical jump landing technique: A randomized controlled trial. Hum Mov Sc. 2016; 45:84-95.

Welling W, Benjaminse A, Seil R, Lemmink K, Zaffagnini S, Gokeler A. Low rates of patients meeting return to sport criteria 9 months after anterior cruciate ligament reconstruction: a prospective longitudinal study. Knee Surg Sports Traumatol Arthrosc. 2018.

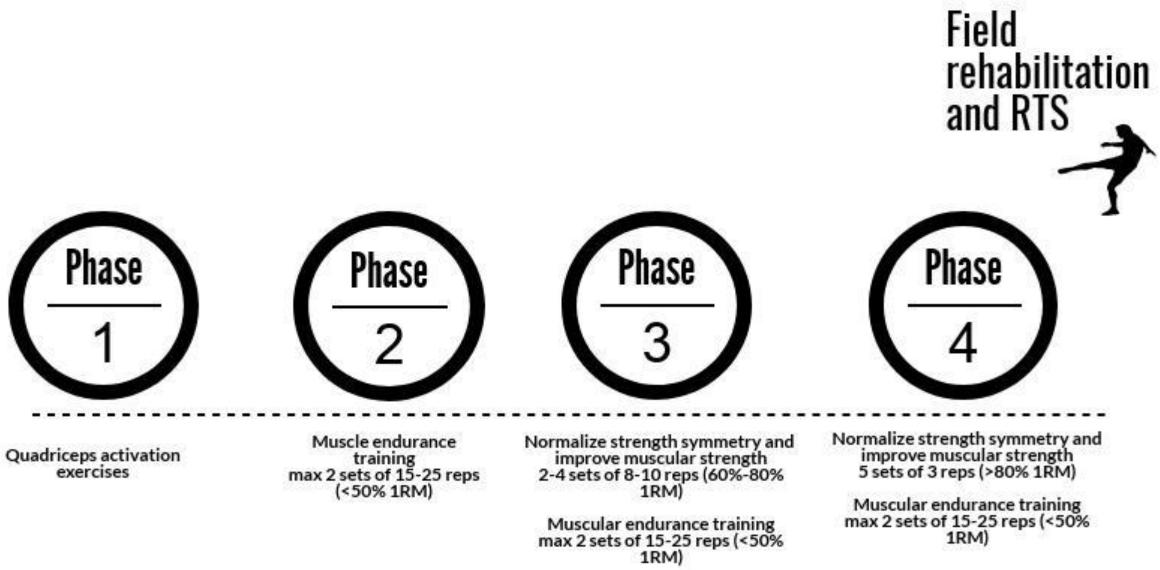

Figure 1. Timeline of the different phases within the strength training protocol, including training parameters. 1RM=one-repetition maximal, RTS=return to sport.

Figure 2. Two examples of open kinetic chain exercises performed during the strength training. 1=knee extension, 2=leg curl.

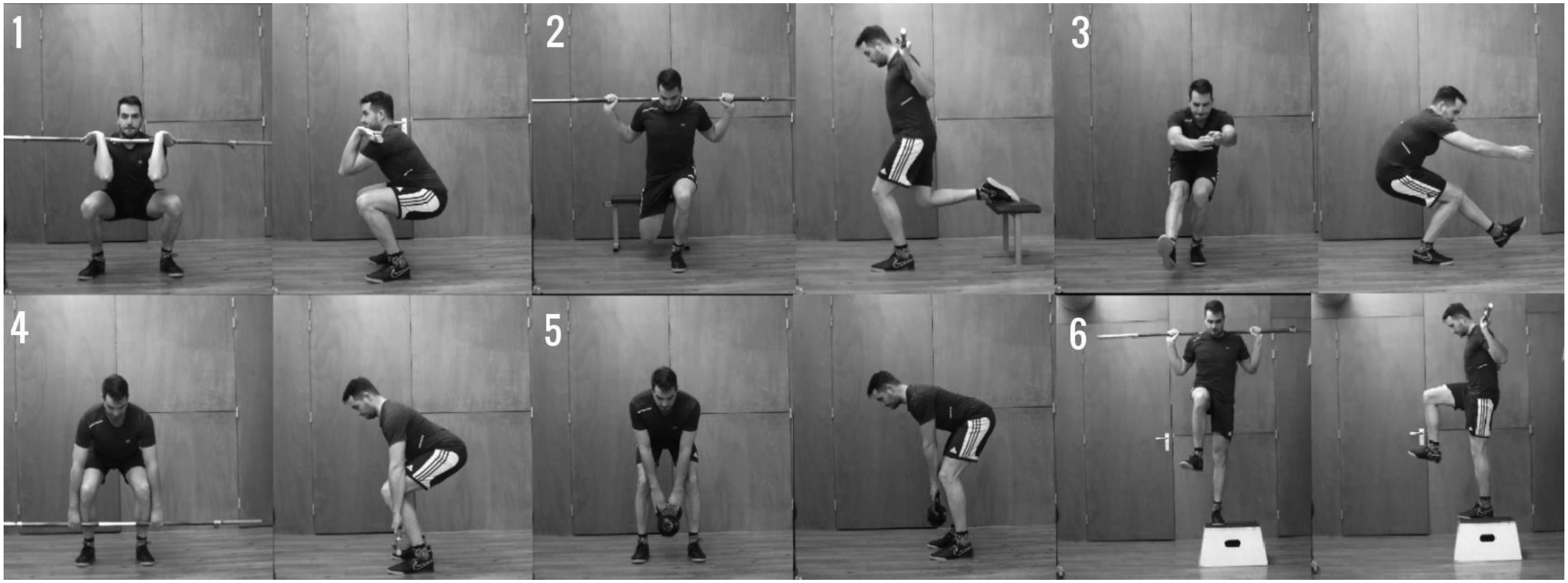

Figure 3. Six examples of one-legged and two-legged closed kinetic chain exercises performed during the strength training. 1=front squat, 2=split squat, 3=pistol squat, 4=dead lift, 5=good morning, 6=step up.

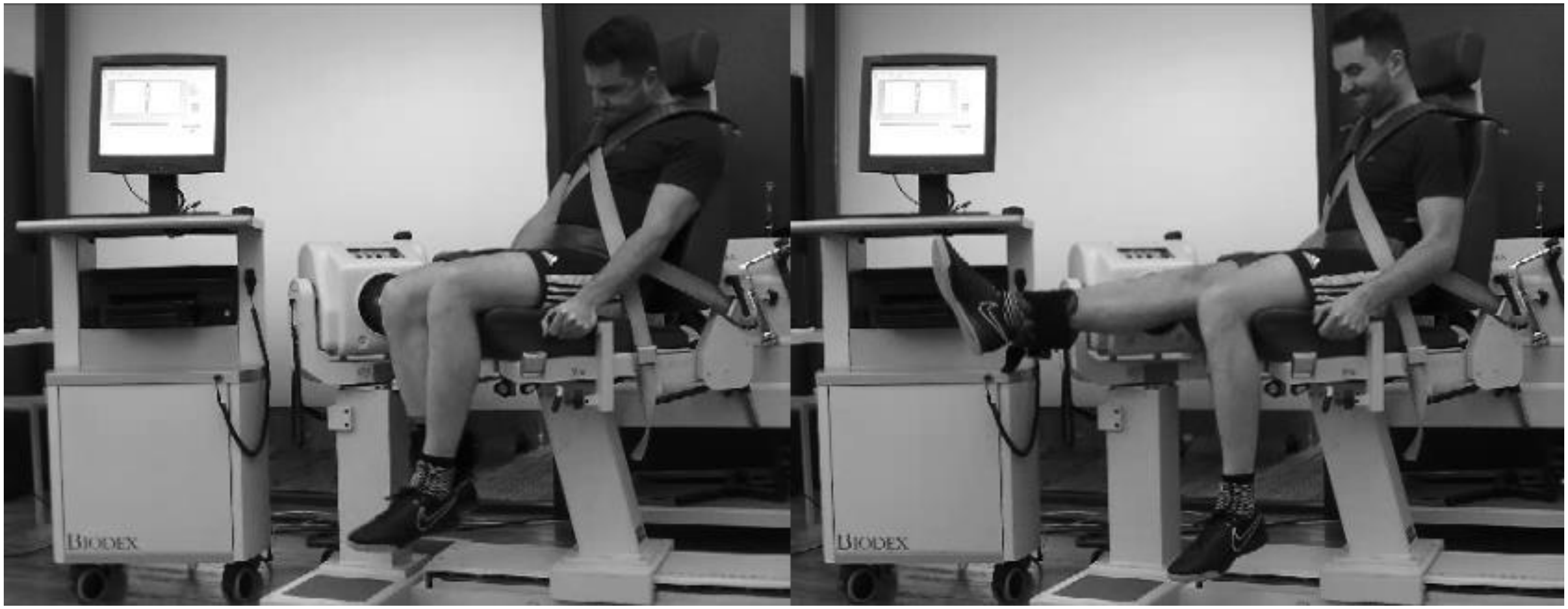

Figure 4. Patient performing a concentric isokinetic strength test at 60°/s for knee flexion (left) and knee extension (right).

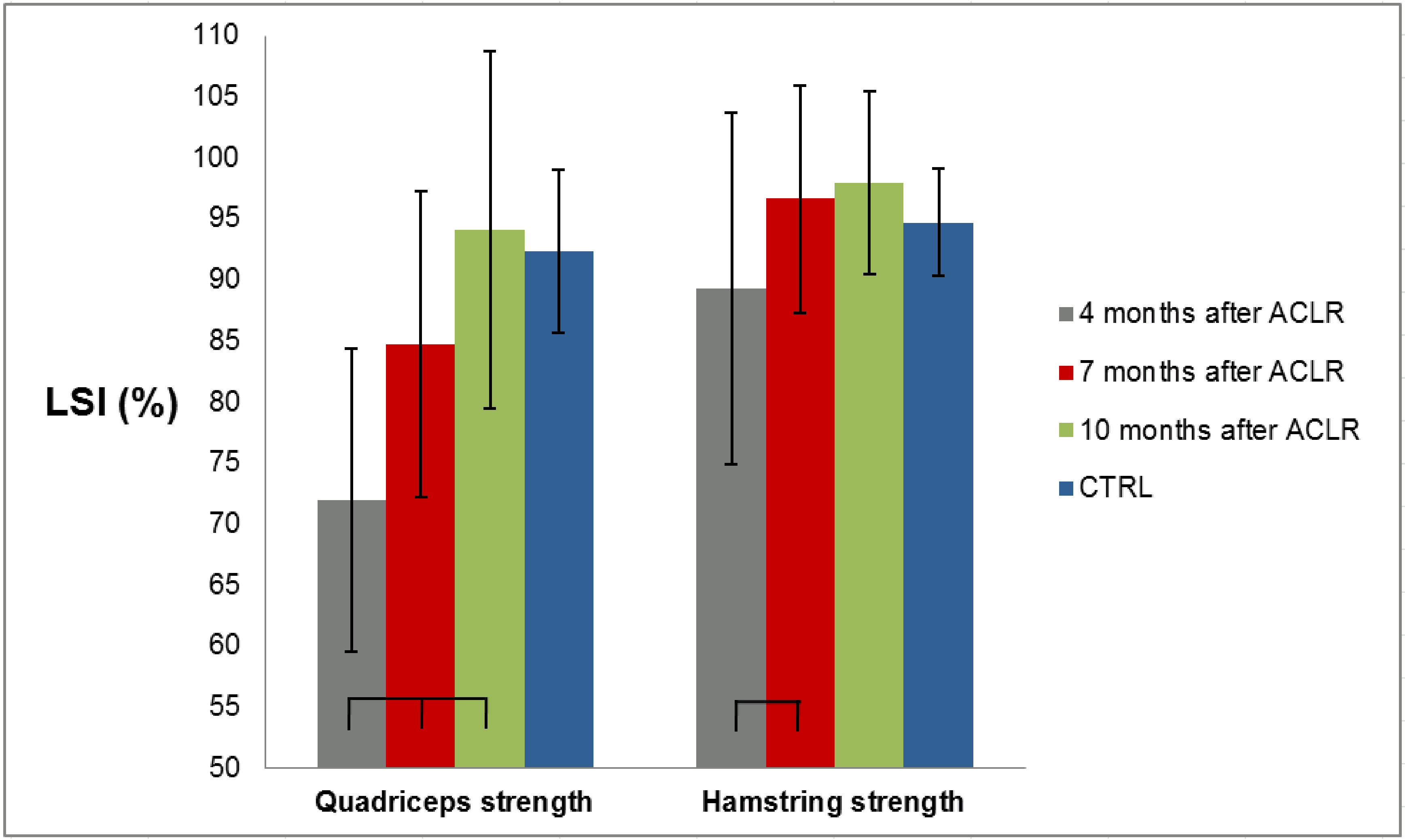

Figure 5. Graphical representation of the Limb Symmetry Index values of the soccer players after ACLR and the control group. LSI=limb symmetry index, ACLR=anterior cruciate ligament reconstruction patients, bracket=significant difference compared to previous measurement ($p<0.05$).

Table 1. Demographic data.

	Soccer players after ACLR	Control group	p-value
Number of subjects (n)	38	30	N.A.
Age (years)	24.2±4.7	22.8±2.5	0.162
Weight (kg)	79.0±13.3	72.7±6.8	0.018*
Tegner Activity Level	9.0±0.0	9.0±0.0	N.A.
Graft type	HT(24), BPTB(14)	N.A.	N.A.
Time after surgery (months)	3.9±1.1[1]; 6.6±0.7[2]; 9.7±0.8[3]	N.A.	N.A.
Injured leg is dominant leg (%)	76.3	N.A.	N.A.
Number of treatments (n)	44.2±9.9[1]; 77.5±13.2[2]; 108.0±15.1[3]	N.A.	N.A.
IKDC	68.0±6.0[1]; 78.0±8.6[2]; 86.5±5.4[3]	N.A.	N.A.

ACLR = anterior cruciate ligament reconstruction, kg=kilogram, HT=hamstring tendon graft, BPTB=bone-patellar tendon graft, 1=at strength test 1, 2=at strength test 2, 3=at strength test 3, IKDC= International Knee Documentation Committee Subjective Knee Form, N.A.=not applicable, *=significant difference ($p<0.05$).

Table 2. Criteria within the rehabilitation protocol.

Activity	**Strength criteria**
Return to running	LSI >70% at 60°/s for both quadriceps and hamstring strength (Rambaud et al., 2018)
Return to sport specific training	Males: PT/BW for quadriceps muscle strength males >1.6 at 180°/s and >1.4 at 300°/s in extension for the injured leg Females: PT/BW for quadriceps muscle strength >1.5 at 180°/s and >1.3 at 300°/s in extension for the injured leg (Myer et al., 2008)
Return to field rehabilitation	LSI >85% at 60°/s, 180°/s and 300°/s for both quadriceps and hamstring strength (Karasel et al., 2010)
Return to sport	LS I>90% at 60°/s, 180°/s and 300 °/s for both quadriceps and hamstring strength PT/BW >3.0 for quadriceps muscle strength at 60°/s in extension for the injured leg H/Q ratio >55% for females and >62.5% for males for the injured leg at 300°/s (Gokeler et al., 2017; Welling et al., 2018)

LSI=limb symmetry index, °/s=degrees per second, PT/BW=peak torque/body weight, H/Q ratio=hamstring/quadriceps ratio.

Table 3. Strength data of the soccer players after ACLR and the control group.

	Group	Leg	Time	Mean±SD	LSI	p-value between legs	p-value over time
Peak quadriceps muscle strength (Nm)	ACLR	Injured	3.9 months	188.6±51.6	72.0±12.4	**<0.001***	N.A.
	ACLR	Non-injured	3.9 months	262.0±57.6	N.A.	N.A.	N.A.
	ACLR	Injured	6.6 months	223.4±51.1	84.7±12.5	**<0.001***	**<0.001***
	ACLR	Non-injured	6.6 months	267.3±57.5	N.A.	N.A.	0.163
	ACLR	Injured	9.7 months	256.7±51.0	94.1±14.6	**0.001***	**<0.001***
	ACLR	Non-injured	9.7 months	269.5±61.0	N.A.	N.A.	0.677
	CTRL	Dominant	N.A.	231.7±27.0	92.3±6.7	**<0.001***	N.A.
	CTRL	Non-dominant	N.A.	217.0±32.2	N.A.	N.A.	N.A.
Peak hamstring muscle strength (Nm)	ACLR	Injured	3.9 months	128.0±31.2	89.3±14.4	**<0.001***	N.A.
	ACLR	Non-injured	3.9 months	143.3±30.6	N.A.	N.A.	N.A.
	ACLR	Injured	6.6 months	143.8±29.9	96.6±9.3	**0.047***	**<0.001***
	ACLR	Non-injured	6.6 months	148.8±34.2	N.A.	N.A.	**0.038***
	ACLR	Injured	9.7 months	149.5±31.2	97.9±7.5	0.521	**0.019***
	ACLR	Non-injured	9.7 months	152.7±34.3	N.A.	N.A.	0.433
	CTRL	Dominant	N.A.	136.3±21.1	94.7±4.4	0.505	N.A.
	CTRL	Non-dominant	N.A.	135.1±20.6	N.A.	N.A.	N.A.

p-value between legs=difference between legs at specific time point, p-value over time=difference compared to previous time point, ACLR=anterior cruciate ligament reconstruction group, CTRL=control group, Nm=newton meter, SD=standard deviation, LSI=limb symmetry index, N.A.=not applicable, *=significant difference (p<0.05).

Table 4. Data of quadriceps peak torque normalized to body weight for the soccer players after ACLR and the control group including percentages of subjects that passed the >3.0 Nm/kg criteria.

	Group	Leg	Time	Mean±SD (Nm/kg)	>3.0 Nm/kg
Peak torque quadriceps muscle strength normalized to bodyweight	ACLR	Injured	3.9 months	2.4±0.5	7.9%
	ACLR	Non-injured	3.9 months	3.3±0.5	65.8%
	ACLR	Injured	6.6 months	2.9±0.5	61.5%
	ACLR	Non-injured	6.6 months	3.3±0.5	84.2%
	ACLR	Injured	9.7 months	3.2±0.6	71.1%
	ACLR	Non-injured	9.7 months	3.4±0.5	89.5%
	CTRL	Dominant	N.A.	3.2±0.3	70.0%
	CTRL	Non-dominant	N.A.	3.0±0.4	50.0%

ACLR=anterior cruciate ligament reconstruction group, CTRL=control group, SD=standard deviation, Nm/kg=Newton meter/kilogram, N.A.=not applicable, *=significant difference (p<0.05).