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Original Article

# Persistent quadriceps muscle atrophy after anterior cruciate ligament reconstruction is associated with alterations in exercise-induced myokine production

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ABSTRACT

*Purpose:* Persistent quadriceps muscle atrophy is observed in a subset of patients following anterior cruciate ligament reconstruction (ACLR) despite the completion of comprehensive rehabilitation. Critically, quadriceps muscle atrophy correlates with muscle weakness and quadriceps strength deficits. The aim of this study was to examine the effect of resistance exercise on myokine levels and muscle atrophy status in ACLR patients with persistent quadriceps muscle atrophy.

*Methods:* Sixteen participants between the ages of 18–39 with a Tegner score of >6 and who had undergone ACLR with hamstring graft were recruited for the study. Quadriceps muscle thicknesses were ascertained by ultrasonography and isokinetic strength assessments were made prior to commencing a single bout of resistance exercise training (RET). Blood samples were taken before and after RET and assayed for myokine expression. Self-reported activity level and knee function questionnaires were completed and recorded.

*Results:* Clustering by quadriceps muscle size measurements created a non-atrophy group of 9 subjects and an atrophy group of 7 subjects. There were no significant between-group differences in anthropometric measurements, time post operation and knee function questionnaires, but the atrophic group comprised of patients with lower pre-injury sporting levels. The atrophy group exhibited significant lower side-to-side muscle thickness ratios and a decreasing trend in quadriceps strength deficits. Serum brain-derived neurotrophic factor (BDNF) was up-regulated in response to RET in non-atrophy group, but a negative fold change was detected in the atrophy group.

*Conclusion:* The dysregulation in myokines plays an important role in patients failing to regain muscle mass after ACLR leading to persistent quadriceps muscle atrophy, which may potentiate greater strength deficits and poor functional recovery.

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Level of evidence IV.

## 1. Introduction

Anterior cruciate ligament (ACL) injury is one of the most common traumatic knee injuries experienced by athletes. ACL reconstruction (ACLR) surgery is the mainstay treatment for restoring knee stabilization and function, which are crucial for athletes to return to pre-injury levels of performance. However, a meta-analysis has demonstrated that up to 35% of patients fail to

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return to their pre-injury level of sport involvement after an ACLR.[1] It is well known that recuperation of quadriceps muscle strength will largely determine whether an athlete returns to sport.[2] Although physical rehabilitation including resistance exercise training (RET) has been implemented to regain muscle mass and strength from post-injury and postoperative quadriceps muscle atrophy, persistent quadriceps atrophy is still common. An average of 7% side-to-side deficit in total quadriceps volume was observed in patients at 6–12 months post ACLR,[3] and a 4% deficit at 12–18 months post ACLR, which corresponded to 9% and 11% side-to-side strength deficits respectively. These figures included both successful and failed cases of muscle regain after physical rehabilitation. Since lower quadriceps muscle mass may contribute to strength deficits after ACLR,[4] it is necessary to identify cases of persistent muscle atrophy for a better management.

The causes of persistent muscle atrophy after ACLR remain unknown. There is a growing interest in understanding the underlying trophic mechanisms whereby exercise stimulates muscle growth and why in some patients an insufficient hypertrophic muscular response is elicited following rehabilitation after ACLR, resulting in persistent quadriceps muscle atrophy. The dysregulation of myokines production and release in response to RE training may provide one possible explanation. Myokines are a collection of trophic, regenerative, metabolic, anti-inflammatory, and immunity-boosting peptide factors that are produced and released by muscle in response to enhanced muscular energy metabolism, such as that required for the execution of exercise. Their interactions can elaborate at the autocrine, paracrine or endocrine levels and their effects are largely adaptive in nature in response to metabolic and physical demands.[5] Some myokines have been implicated in regulating muscle volume. Notably, muscle hypertrophy is suppressed by myostatin, whose initially elevated levels descend back to baseline levels during the postoperative period after ACLR.[6] Brain-derived neurotrophic factor (BDNF) has been implicated in the regulation of myogenesis and muscle regeneration after injury.[7] An altered myokine response to exercise may lead to either high levels of myokines suppressive of muscle hypertrophy, or insufficient levels of hypertrophy-inducing myokines, or a combination of both, resulting in persistent muscle atrophy during rehabilitation of ACLR.

In this study, we aimed at comparing the changes in serum levels of myokines after RET, in ACL-reconstructed patients with persistent quadriceps muscle atrophy as compared to those without muscle atrophy. As the magnitude of changes in muscle atrophy may vary across different quadriceps muscles,[8] patients with persistent muscle atrophy were classified by cluster analysis based on measurements of individual quadriceps muscles. We hypothesized that exercise-induced myokines regulation would be disrupted in patients with persistent quadriceps muscle atrophy, manifested as blunted myokines responses to RET.

## 2. Materials and methods

### 2.1. Ethics

The study was approved by authors' institution (ethics info blinded for review purpose), and in compliance with the Declaration of Helsinki and ICH-GCP. Patients have given written consent to participate in the study.

### 2.2. Study design

A cross-sectional case series analysis of serum myokine levels was performed in patients that had received an ACLR. Patients undergoing a unilateral ACLR with hamstring graft were recruited for the study from the authors' institution. All recruited patients had a Tegner activity score of 6 or greater before the injury. The exclusion criteria for participation in the study included any of the following: 1) a history of injury on the contralateral knee; 2) concomitant bone fracture; 3) meniscus injury requiring altered rehabilitation program; 4) full-thickness chondral injuries and; 5) preoperative radiographic signs of arthritis, metal implants or non-hamstring graft for ACLR. All patients completed a standardized rehabilitation program at the same outpatient physiotherapy clinic, commencing within the first two weeks post-ACLR. Assisted gait training and passive range of motion exercises were assigned in the first four weeks to control pain and swelling, as well as to develop muscular control. Therapeutic exercise in ascending intensity was performed from four to ten weeks post-ACLR to re-establish muscle strength, including whole-body vibration therapy with squats, leg press, stationary cycling, and balance exercises. At ten weeks, patients were assigned running, jumping and agility tasks to complement muscle hypertrophy. From the sixth month onwards, patients were allowed to gradually return to sports under strict supervision. The patients were considered to have completed the rehabilitation program after a full functional assessment at 6–9 months post-operatively, depending on the progress and functional demand.

### 2.3. Resistance exercise training (RET) protocol

All patients were requested to restrain from any heavy exercise 48 h before the exercise test for myokine measurement. The RE training protocol used in this study was shown to be effective in modulating the serum myokine levels immediately and 24 h after the exercise stimulus.[9] Each patient was asked to perform a single session of bilateral lower-limb resistance exercises, consisting of four sets of 8–15 repetitions on the leg press machine and then on a knee extension machine. Each repetition was assessed at 75% of one-repetition maximum strength and each set was performed to task failure. A preliminary assessment of maximum strength level was completed two weeks prior to the actual exercise test. All patients were instructed to lower the weights slowly during the eccentric phase of each repetition, while rapidly pushing the weights up during the concentric phase. There was a 90-s rest period between each set and a 3-min rest period between the two machines. The complete exercise session (a total of eight sets) was completed in ~20 min.

### 2.4. Measurements

#### 2.4.1. Patient reported outcome measures

Patients were asked to complete three patient-reported questionnaires to evaluate the knee function and activity level 6–9 months after the ACL reconstruction. The International Knee Documentation Committee (IKDC) Subjective Knee Form ascertained the patient's ability to perform daily-tasks, symptoms, and knee function.[10] The Tegner Activity Scale was designed to assess activity levels relevant to sports performance, while the Lysholm Knee Score examines knee-specific symptoms and functionality with respect to daily life.[11]

#### 2.4.2. Isokinetic muscle strength

The Biodex System 4 isokinetic dynamometer (Biodex Medical Systems Inc., New York, USA) was used to measure quadriceps muscle strength. The measurement was performed at the same time with other functional assessment questionnaires, at 6–9 months post operatively. Patients were first asked to warm up on a cycling machine for 5 min at their own pace. They were then seated on the dynamometer chair and secured with straps across the

chest, pelvis, and thigh, before performing three submaximal voluntary concentric contractions on both legs for familiarization purposes. To determine the range of motion of the knee joint patients were instructed to do one maximal knee flexion and extension. Bilateral isokinetic knee extension and flexion were tested at 60°/seconds for five repetitions and 180°/seconds for 10 repetitions.[12] Peak torque normalized to body weight (Peak TQ/BW) were used to determine strength deficit as revealed by the ratio of the involved and uninvolved limbs.

#### 2.4.3. Ultrasonographic measurement of quadriceps muscle thickness

The Aixplorer® ultrasound system (SuperSonic Imagine, Aix-en-Provence, France) and a linear transducer probe with a bandwidth of 2–10 MHz (SuperLinear™ SL10-2, Vermon, Tours, France) were used to measure the thickness of the Vastus Medialis (VM), Vastus Lateralis (VL), and Rectus Femoris (RF) muscles on both the involved and uninvolved legs. The ultrasonographic measurement was performed at the same time with other functional assessments, at 6–9 months post operatively. Patients were asked to lay supine on the treatment table for the assessment. A measuring tape was used to locate VM, VL, RF and the patella by palpation, consequently marked with a pen for reference (Fig. 1). For consistency of measurement and ease of comparison across patients, the locations of the three muscle groups were assigned using the following guidelines: 1) RF was marked at 1/2 of the distance from the anterior superior iliac spine (ASIS) to the superior pole of the patella; 2) VM was located at 1/5 of the distance away from the midpoint of the medial patella border to the ASIS and; 3) VL was denoted at 1/3 of the distance from the midpoint of the lateral patella border to the ASIS. An excess of contact gel was next applied to the located appropriate anatomical points. The transducer probe was aligned in the transverse plane and moved along the entire muscle bundle to capture a complete view of the VM, VL and RF (Fig. 2). The probe was then positioned in the sagittal plane of the muscle to determine its thickness. Minimal pressure was applied through the probe onto the limb to prevent the deformation of the underlying musculature. The thickness was measured as average of three measurements. Muscle thickness ratio was calculated by dividing the muscle thickness of the involved (injured) limb by that of the uninvolved (contralateral) limb.

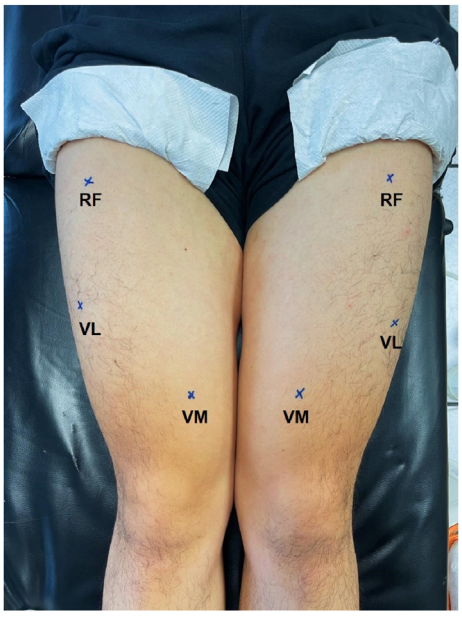


**Fig. 1.** Vastus Medialis (VM), Vastus Lateralis (VL), and Rectus Femoris (RF) muscles on both the involved and uninvolved legs were marked for ultrasonographic measurement.

#### 2.4.4. Serum myokine measurements

Blood samples of 5 mL were taken immediately before and within 30 min of having completed the RE session. The samples were left coagulated at room temperature for 15–30 min, followed by centrifugation at 2000*g* for 10 min at 4 °C. After centrifugation, the serum was aliquoted and stored at −80 °C for subsequent analysis. Serum myokine levels were analyzed by MILLIPLEX® Map Human Myokine Magnetic Bead Panel (HMYOMAG-56 K Millipore, Billerica, MA) employing a Bioplex-200 system (BioRad, Hercules, CA), including measurement of Apelin, Brain-derived neurotrophic factor (BDNF), Erythropoietin (EPO), Fatty Acid Binding Protein 3 (FABP3), Fibroblast growth factor 21 (FGF-21), Fractalkine (CX3CL1), Follistatin-like Protein 1 (FSTL1), Interleukin 6 (IL-6), Interleukin 15 (IL-15), Irisin, Leukemia inhibitory factor (LIF), Myostatin (GDF8), Oncostatin M (OSM), Osteocrin (Musclin, OSTN), Osteonectin (SPARC), according to the manufacturer's instructions. Fold change was calculated by dividing post-exercise serum myokine concentration by pre-exercise levels.

#### 2.4.5. Statistical analysis

Due to the lack of a gold standard of cut-off values for muscle atrophy with respect to muscle thickness, this study performed a cluster analysis based on the muscle thickness ratio to distinguish atrophy and the non-atrophy group. In brief, two clusters were defined by setting initial cluster centers according to maximum and minimum values in muscle thickness ratios in RF, VL and VM. Iterations and classifications of cases were performed until convergence was achieved with minimal changes in cluster centers. The association between serum levels of myokines, quadriceps muscle atrophy, quadriceps muscle strength, knee function and return-to-play were checked by bivariate correlation analysis. For the main analyses, the fold changes in serum levels of myokines after RE and the differences between patients with and without muscle atrophy after ACLR were tested by One-Sample *t*-Test and Mann-Whitney *U* test, respectively. The level of significance was set at $\alpha = 0.05$. All the statistical tests were performed using the Statistical Package for Social Sciences (IBM SPSS Ver. 24).

## 3. Results

A total of sixteen patients, eight males and eight females participated in the study. The baseline characteristics of the subjects are shown in Table 1.

Significant quadriceps muscle atrophy and strength deficit were evident in this group of ACLR patients, as shown by decreased quadriceps muscle thickness ratio and peak torque ratio. The ratio of RF thickness was not different from 1 (CI:0.929, 1.016), but low ratios of VM (CI:0.712, 0.914) and VL (CI: 0.792, 0.978) thickness contributed to the quadriceps muscle atrophy. Isokinetic strength assessments, at both 60 and 180° per second, revealed around 26% strength deficits as shown by peak torque ratios, which showed significant association with muscle thickness ratio of VM (Spearman Rho = 0.796, $p < 0.001$). Significant decrease in activity level after ACLR was noticed by a decreased Tegner score as compared to pre-injury level (Wilcoxon signed rank test, $p = 0.011$), and 50% of patients were unable to return to pre-injury activity levels. The change in activity level was correlated with poor IKDC score (Spearman Rho = 0.774, $p = 0.005$), but not with muscle atrophy and strength deficits. IKDC score was significantly correlated with peak torque ratio (Spearman Rho = 0.659, $p = 0.001$) and

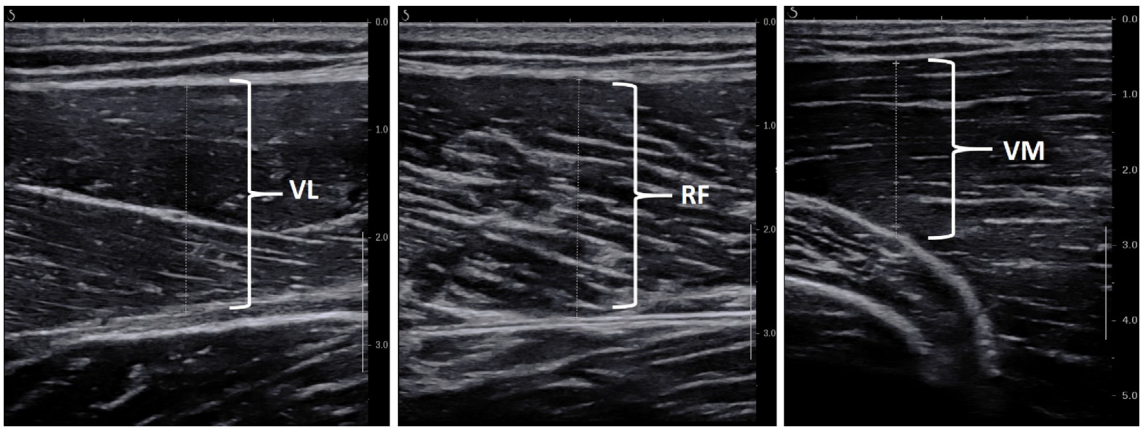


**Fig. 2.** Sagittal plane of Vastus Medialis (VM), Vastus Lateralis (VL), and Rectus Femoris (RF) muscles for ultrasonographic measurement of muscle thickness.

**Table 1**
Baseline characteristics of recruited subjects with respect to demographics, patient-reported outcome measures, ultrasonographic measurement of quadriceps thickness and isokinetic muscle strength.

| | | | |
|---|---|---|---|
| Number of subjects (Number of female) | | 16(8) | |
| Age (years) | | 25.7 ± 6 | |
| BMI (kg/m$^2$) | | 22.0 ± 26 | |
| Time Post-Op (months) | | 17.9 ± 10 | |
| IKDC Score | | 82(22) | |
| Lysholm Score | | 88 (44) | |
| Tegner Score | | *Before injury* | 7(5) |
| | | *After ACLR* | 6.5±5 |
| | | *Difference* | -1(3) |
| RF (cm) | *Uninvolved* | *Involved* | *Ratio* |
| | 2.35 ± 0.4 | 2.28 ± 0.3 | 0.973 ± 0.08 |
| VM (cm) | *Uninvolved* | *Involved* | *Ratio* |
| | 2.54 ± 0.5 | 2.04 ± 0.5 | 0.812 ± 0.19 |
| VL (cm) | *Uninvolved* | *Involved* | *Ratio* |
| | 2.25 ± 0.5 | 1.97 ± 0.4 | 0.886 ± 0.17 |
| Extension Peak TQ/BW at 60°/s (%) | *Uninvolved* | *Involved* | *Ratio* |
| | 248.0 ± 60 | 183.0 ± 60 | 0.741 ± 0.19 |
| Extension Peak TQ/BW at 180°/s (%) | *Uninvolved* | *Involved* | *Ratio* |
| | 181.5 ± 34 | 137.0 ± 42 | 0.747 ± 0.15 |
| Flexion Peak TQ/BW at 60°/s (%) | *Uninvolved* | *Involved* | *Ratio* |
| | 109.8 ± 34 | 97.3 ± 30 | 0.891 ± 0.11 |
| Flexion Peak TQ/BW at 180°/s (%) | *Uninvolved* | *Involved* | *Ratio* |
| | 80.4 ± 21 | 77.5 ± 23 | 0.966 ± 0.12 |
| H/Q ratio at 60°/s (%) | *Uninvolved* | *Involved* | |
| | 45.0 ± 12 | 55.6 ± 18 | |
| H/Q ratio at 180°/s (%) | *Uninvolved* | *Involved* | |
| | 44.0 ± 8 | 58.5 ± 16 | |

*TQ = Torque; BW = Body Weight; H/Q = Hamstrings strength/Quadriceps strength.

muscle thickness ratio in VM (Spearman Rho = 0.520, p = 0.039). Time post operation did not correlate with all PROM, muscle thickness ratios and peak torque ratios.

The clustering of thickness ratios for the RF, VM, and VL muscles was shown in Fig. 3. Cluster analysis resulted in 9 non-atrophic and 7 atrophic patients, with cluster centers close to 1 in muscle thickness ratios in the non-atrophy group (Table 2), indicating limb symmetry in quadriceps muscle size. In contrast, significant asymmetry was observed in VL and VM in the atrophy group, in which around 30% strength deficit was entailed. Only 20% strength deficits were observed in the non-atrophy group. There were no significant differences in peak torque ratio (p = 0.153) and peak torques (involved: p = 0.315, uninvolved: p = 0.711) between non-atrophy and atrophy groups.

There were no significant differences in age, sex ratio, BMI, time post operation, IKDC, Lysholm and Tegner scores after ACLR between non-atrophy and atrophy groups (Table 3); but significantly lower pre-injury Tegner score was observed in the atrophy group (Mann-Whitney test, p = 0.021).

Brain-derived neurotrophic factor (BDNF), heart-type fatty acid-binding protein (FABP3), and osteonectin/secreted protein acidic and rich in cysteine (SPARC) were the only myokines detected in all samples for our serum panel analysis. The concentrations and fold changes for BDNF, FABP3, and SPARC between atrophy (blue) and non-atrophy (red) groups are given in Fig. 4 and depicted in Table 4.

All patients demonstrated significant fold change (p = 0.001) in exercise-induced release of FABP3, whereas BDNF (p = 0.918) and SPARC (p = 0.379) did not. When atrophy group was compared to non-atrophy group, FABP3 displayed consistent trends being upregulated, but BDNF gave significant opposing responses (p = 0.023) to acute exercise as negative (atrophy) and positive fold changes (non-atrophy).

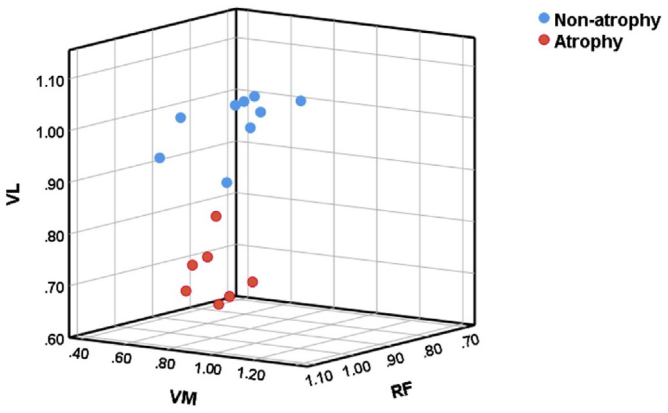


**Fig. 3.** 3D scatter plot of muscle group thickness ratio distribution in the clustered groups assigned as non-atrophy (muscle thickness ratio ~1) and atrophy group (muscle thickness ratio <1).

## 4. Discussion

We demonstrate an association between persistent muscle atrophy and deranged exercise-induced myokine production amongst patients receiving ACL reconstruction (ACLR patients). It is well-known that ACLR patients experienced significant quadriceps atrophy within the first 30 weeks post-surgery.[13] With rehabilitative exercise implemented after knee stability was restored (usually 3 months post-surgery), muscle size would be regained and only moderate side-to-side deficits were noticed from 52 weeks onwards.[13] However, some ACLR patients were unresponsive to physical rehabilitation and retained the quadriceps muscle atrophy, which may account for the failure to return to play. Our findings suggest that the deranged exercise-induced muscle hypertrophy may be attributed to altered myokine response. It calls on further improvement on rehabilitation program to facilitate regain of quadriceps mass and strength by securing a normal myokine response to physical exercise.

In contrast to the existing studies that focused on measuring the muscle volume or cross-sectional area (CSA) by MRI, this study utilized ultrasound imaging to measure muscle thickness, which is more convenient but may be less accurate. According to a systematic review and meta-analysis,[13] CSA measurement only detected gross quadriceps muscle atrophy but failed to reveal difference in RF, VL and VM; while volumetric measurement reported significant side-to-side difference in quadriceps, VL and VM; but not RF. Our findings are similar to the reports with volumetric measurements. It might be due to the appropriate choices in region of interest for ultrasonographic measurement for muscle thickness, which covered the belly regions of RF, VL and VM, that located at different positions along the thigh. On the contrary, measurements by CSA at the same thigh position may not be sensitive enough to detect subtle changes in individual quadriceps muscles. Quantitatively, it is reported[13] that the Cohen's d of side-to-side deficits in quadriceps muscle volume for VM and VL at 28 weeks post-ACLR were −0.44 and −1.06 respectively; while our data revealed a 19% (VM) and 12% (VL) side-to-side deficits, which corresponded to Cohen's d as −0.62 (VM) and −0.95 (VL). It suggests that the use of ultrasonographic measurement of muscle thickness may achieve similar sensitivity to detect quadriceps muscle atrophy by volumetric measurements.

In this case series, 50% of patients were unable to return to pre-injury activity levels and large variations in the extent of quadriceps muscle atrophy were evident. It is obvious that some ACLR patients did not recover satisfactorily after surgery and rehabilitation. Persistent quadriceps atrophy was correlated to significant strength deficits, which may partially account for poor knee functions and failed return to play.[14] However, it is difficult to identify cases of muscle atrophy without validated cut-off values in muscle size measurements. Based on muscle thickness ratios of RF, VM, and VL, we objectively identified a group of ACLR patients with persistent muscle atrophy by cluster analysis. Among different quadriceps muscles, atrophic changes in VL and VM were more prominent, whereas RF was relatively less affected. Establishment of cut-off values can rely on the boundary values from these initial clusters, a combination of muscle size measurements in VL, VM and RF can

**Table 2**
Muscle thickness measurements and isokinetic muscle strengths of uninvolved and involved limbs in clustered groups.

| Muscle thickness measurements and isokinetic muscle strengths | Non-atrophy group N = 9 | | | Atrophy group N = 7 | | |
|---|---|---|---|---|---|---|
| | ***Uninvolved*** | ***Involved*** | ***Ratio*** | ***Uninvolved*** | ***Involved*** | ***Ratio*** |
| RF | 2.35 ± 0.3 | 2.37 ± 0.3 | 1.01 ± 0.2 | 2.35 ± 0.3 | 2.16 ± 0.3 | 0.922 ± 0.08* |
| VM | 2.38 ± 0.3 | 2.20 ± 0.5 | 0.93 ± 0.1 | 2.75 ± 0.5 | 1.83 ± 0.4 | 0.668 ± 0.11* |
| VL | 2.11 ± 0.4 | 2.17 ± 0.4 | 1.03 ± 0.1 | 2.44 ± 0.5 | 1.71 ± 0.3 | 0.706 ± 0.07* |
| Extension Peak TQ/BW at 60°/s | 243 ± 53 | 196 ± 61 | 0.810 ± 0.19 | 256 ± 72 | 166 ± 58 | 0.652 ± 0.17 |
| Flexion Peak TQ/BW at 60°/s | 120 ± 28 | 107 ± 25 | 0.898 ± 0.12 | 97 ± 38 | 86 ± 34 | 0.882 ± 0.10 |
| HQ ratio at 60°/s | 50 ± 10 | 58 ± 17 | N.A. | 38 ± 12* | 53 ± 22 | N.A. |
| Extension Peak TQ/BW at 180°/s | 177 ± 30 | 137 ± 39 | 0.769 ± 0.13 | 188 ± 40 | 137 ± 48 | 0.718 ± 0.17 |
| Flexion Peak TQ/BW at 180°/s | 83 ± 18 | 80 ± 24 | 0.956 ± 0.14 | 77 ± 26 | 74 ± 23 | 0.979 ± 0.09 |
| HQ ratio 180°/s | 47 ± 6 | 59 ± 14 | N.A. | 40 ± 9 | 57 ± 19 | N.A. |

**Table 3**
Descriptive Statistics of patient demographics and patient self-reported outcome measures in clustered groups.

| Patient demographics and patient self-reported outcome measures | | Non-atrophy group N = 9 (6 female) | Atrophy group N = 7 (2 female) |
|---|---|---|---|
| Age (years) | | 25.2 ± 4 | 26.3 ± 5 |
| BMI (kg/m$^2$) | | 22.3 ± 1 | 22.3 ± 2 |
| Time Post-Op (months) | | 15.7 ± 6 | 20.9 ± 8 |
| IKDC Score | | 87 (25) | 80.5 (24) |
| Lysholm Score | | 90 (28) | 86(11) |
| Tegner | Before Injury | 9 (2.5) | 7(1)* |
| | After ACLR | 5(5) | 7(3) |
| | Differences | −2[4] | (3) |

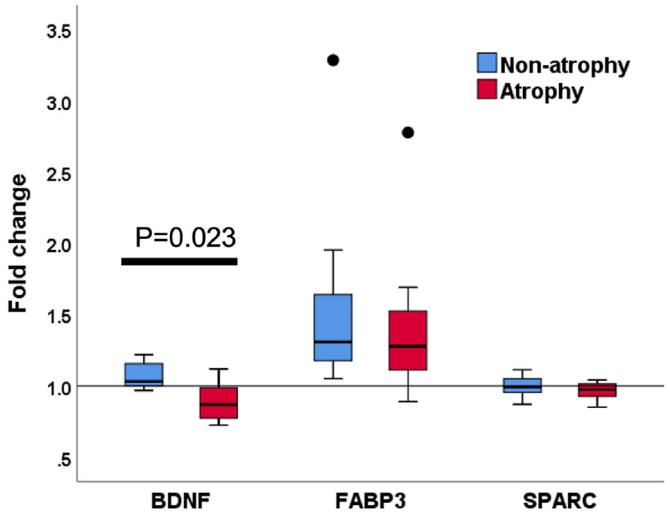


**Fig. 4.** Fold change of myokines after resistance exercise training in clustered groups. Significant difference in BDNF fold change was detected.

**Table 4**
Serum levels of myokine (in pg/mL) detected before and after single bout of resistance exercise training in the clustered groups.

| Myokine | | Non-atrophy group N = 9 | Atrophy group N = 7 |
|---|---|---|---|
| BDNF | Before exercise | 12313 ± 4775 | 14999 ± 5745 |
| | After exercise | 13052 ± 4505 | 13815 ± 6314 |
| | Fold change | 1.08 ± 0.1 | 0.891 ± 0.1* |
| FABP3 | Before exercise | 1074 ± 403 | 1284 ± 622 |
| | After exercise | 1665 ± 800 | 1634 ± 791 |
| | Fold change | 1.58 ± 0.7 | 1.46 ± 0.6 |
| SPARC | Before exercise | 450 ± 137 | 356 ± 118 |
| | After exercise | 451 ± 159 | 342 ± 117 |
| | Fold change | 0.995 ± 0.1 | 0.965 ± 0.1 |

then be used to generate an integrative cut-off value by discriminant analysis, which can handle the situations when "atrophic" status in different quadriceps muscles vary.

Side-to-side knee extension strength deficits (extension peak TQ/BW at 60°/s) was noticed in both atrophy (~19%) and non-atrophy groups (~35%) ($p = 0.021$, 0.018 respectively). However, between-group difference in strength deficits was not significant ($p = 0.103$), probably due to insufficient statistical power with a small sample size. Except the pre-injury Tegner score, the atrophy group did not show significant decrease as compared to the non-atrophic group in other PROM (post-op Tegner, IKDC and Lysholm). Functional recovery and return to play after ACLR is not only affected by quadriceps atrophy, but also other factors such as knee stability, neuromuscular control and psychological readiness.

The causes of persistent quadriceps atrophy remain speculative. It is possible that some ACLR patients did not respond ideally to rehabilitation, leading to development of persistent muscle atrophy. Normally, expression of myokines is triggered to mediate adaptive responses to exercise, including muscle hypertrophy. Nonetheless, altered myokine expressions has been demonstrated in several muscle wasting disorders such as diabetes, cancer, and ageing.[15] In this study, serum FABP3 levels increased following a single-bout of resistance exercise, regardless of the degree of muscle atrophy. It is consistent with previous reports that FABP3 expression level was upregulated by physical training.[16] By contrast, the serum levels of BDNF and SPARC were largely unaltered by the exercise stimulus in this cohort of patients. FABP3 enhanced fatty acid metabolism[17] and SPARC is implicated in wound healing and tissue repair,[18] while BDNF was shown to regulate myogenesis and muscle regeneration.[7,19] We report that the extent of muscle atrophy was correlated with altered exercise-induced release of BDNF. BDNF expression has been shown to increase in response to muscle contraction.[20] BDNF expression is upregulated during the activation and proliferation of satellite cells.[21] As BNDF displayed a negative fold change in the atrophy group, it might account for the failed response to rehabilitative exercise for muscle hypertrophy. However, due to the cross-sectional nature of the study design, it is unknown whether the dysregulated response was a cause or consequence of muscle atrophy.

Other possible factors influencing the myokine response would be compliance to the rehabilitation exercise post-operation, diet, and genetics. Yet, our study demonstrated a relationship between the extent of muscle atrophy and pre-injury levels of sporting activities as shown by Tegner scores. The non-atrophy group comprised of professional athletes (Tegner score 9), while the atrophy group mainly played recreational sports (Tegner score 7). It is likely that professional athletes may have higher compliance to rehabilitative exercise post ACLR, resulting in a better regain of muscle mass and function. With the observed alterations in myokine responses, we speculate that training history may also modulate the regulation of exercise-induced myokine expression. The presented findings confirm the importance of investigating the underlying mechanisms accounting for these varied responses to exercise-induced muscle hypertrophy. This can potentially change our current approach for rehabilitation after ACLR as the dysregulated myokine expression specifically observed in the atrophy group may render rehabilitation exercises futile. Different rehabilitation interventions could be developed that are capable of stimulating the production of muscle-hypertrophic myokines or inhibiting the muscle-atrophic ones in the patients, such as biophysical interventions including vibration[12] and pulse-electromagnetic fields.[22] Further studies could focus on investigating potential interventions for ACLR patients that are not as responsive to conventional rehabilitation to prevent persistent quadriceps muscle atrophy.

Certain limitations of the present study are acknowledged. Firstly, this pilot trial was insufficiently powered for the indications measure given the small cohort sizes and large variability in muscle atrophy exhibited amongst the patient cohorts. The introduction of a healthy cohort to serve a reference for "healthy" exercise-induced myokine responses would have benefitted the study. Another limitation of the study was the inability to detect other myokines, particularly those involved with muscle mass regulation such as myostatin and interleukin-15, and could be related to thermosensitivity (sample handling, freeze-thaw cycle), nature of exercise (aerobic or resistance) stimulus, and timing of sample collection not coinciding with peak concentration (immediate or after hours/days). Additionally, some myokines may be preferentially detected with other technologies such as mass spectrometry.[23]

## 5. Conclusion

Persistent quadriceps muscle atrophy after anterior cruciate ligament reconstruction is associated with alterations in exercise-induced myokine production, which may account for the failed response to rehabilitative exercise for muscle hypertrophy.

## Data availability statement

All relevant data is contained within the article: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

## Funding

There was no funding received for this study.

## Ethical approval

The study was approved by The Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (IRB/REC reference number: 2018.604).

## Informed consent

All subjects have given written consent to participate in the study.

## Authors' contribution

MTO and SCF conceived and designed the study. SWM and SLY collected the data. SCF analyzed the data and AFO aided in interpreting the results. MTO and SCF drafted the manuscript with input from all authors and AFO revised critically. MTO and PSY supervised the whole study. All authors approved the final manuscript.

## Declaration of competing interest

All authors declare no conflicts of interest.

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## Abbreviations

| | |
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| ACLR | Anterior cruciate ligament reconstruction |
| ASIS | Anterior superior iliac spine |
| BDNF | Brain-derived neurotrophic factor |
| BMI | Body mass index |
| BW | Body weight |
| CI | Confidence interval |
| CSA | Cross-sectional area |
| CX3CL1 | Fractalkine |
| EPO | Erythropoietin |
| FABP3 | Fatty Acid Binding Protein 3 |
| FGF-21 | Fibroblast growth factor 21 |
| FSTL1 | Follistatin-like Protein 1 |
| GDF8 | Myostatin |
| H/Q | Hamstrings strength/Quadriceps strength |
| ICH-GCP | International Conference on Harmonisation-Good Clinical Practice |
| IKDC | International Knee Documentation Committee |
| IL-6 | Interleukin 6 |
| IL-15 | Interleukin 15 |
| LIF | Leukemia inhibitory factor |
| MRI | Magnetic resonance imaging |
| PROM | Patient-reported outcome measure |
| OSM | Oncostatin M |
| OSTN | Osteocrin (Musclin) |
| RET | Resistance exercise training |
| RF | Rectus Femoris |
| SPARC | Secreted protein acidic and rich in cysteine (Osteonectin) |
| TQ | Torque |
| VL | Vastus Lateralis |
| VM | Vastus Medialis |

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