Vitamin D as an Intervention for Improving Quadriceps Muscle Strength in Patients after Anterior Cruciate Ligament Reconstruction: A Randomized Double-Blinded, Placebo-Controlled Clinical Trial

Background

In Hong Kong, over 3000 Anterior Cruciate Ligament reconstructions (ACLR) are performed each year in order to restore the knee function after an ACL injury. The goal of the ACLR especially for athletes is to return to sports and recondition the athlete back to the preinjury level of sport. Despite a successful surgery and a strenuous rehabilitation process, some athletes still fail to qualify the Return-To-Play (RTP) criteria, and 23% among those who returned to high-risk sport would suffer a second ACL injury (1).

After ACLR, the patient would undergo post-operative rehabilitation to strengthen muscle groups associated to the knee. In the beginning of phase one (0-4 weeks post-surgery), controlled mobilization is required for the protection of the ACL graft and the reduction of swelling. Then, phase 2 (5 - 9 weeks post-surgery) consists of controlled rehabilitative training while phase 3 (10-16 weeks post-surgery) involves intensive muscle strengthening and training. During phase 4 (17-26 weeks post-surgery), patients would undergo advanced agility and endurance training. Finally, phase 5 (26 weeks post-surgery onwards) aims to prepare patients for a gradual return to sports. Most of the patients would be expected to RTP by 12 months. Despite the comprehensive rehabilitation program, a systematic review showed that up to 35% of patients would fail to return to their preinjury level of sport (2).

Quadriceps muscle weakness after ACLR is a major limiting factor for functional recovery. It is contributed by arthrogenic muscle inhibition and muscle atrophy, which are resulted from muscle disuse, joint swelling, pain, inflammation and damage of neuroreceptors in the joint after the surgery. Muscle size is a determinant of muscle strength. Postoperative quadriceps muscle atrophy is inevitable, but muscle strength should be regained through rehabilitation. As the patient continues to exercise during rehabilitation, the atrophic response subsides and the strengthening training stimulates significant hypertrophy in quadriceps muscle, and thus increasing the quadriceps strength (3). However, in some patients, this hypertrophic response is insufficient and can lead to persistent quadriceps muscle atrophy after ACLR.

This study would benefit patients who suffered from persistent quadriceps weakness and atrophy after ACLR, thus improving the post-operative outcome. This would lead to a better RTP rate as well as reducing the risk of reinjury, which can be a burden to the health system and the society due to the additional treatments and extended time off-work.

Objectives

1. To determine the correlation between vitamin D status and quadriceps muscle strength at pre-op and at 4 months after ACLR.
2. To conduct a randomized-controlled trial to examine the therapeutic effects of vitamin D supplement on improving vitamin D deficiency status in patients with quadriceps muscle weakness after ACLR.
3. To examine the effect of vitamin D supplements on the quadriceps muscle strength for patients after ACLR.

Hypothesis

1. Vitamin D supplements will improve the vitamin D status of patients with vitamin D <20ng/ml and quadriceps muscle weakness after ACLR
2. The improved level of vitamin D will lead to an improvement in quadriceps muscle strength and size.

Patient Recruitment & Eligibility

This is a prospective randomized, double-blinded placebo-controlled trial with all participants being recruited at the out-patient clinic of Prince of Wales Hospital. Participants are randomly assigned into either the Vitamin D intervention group or the placebo-controlled group through an online computer generator sequence at the time of admission to the out-patient clinic. 60 participants will be recruited, with 30 in each group. The study will be carried out in compliance with Declaration of Helsinki and the ICH-GCP. Ethical approval will be obtained from the local IRB before the study starts. Informed consent must be obtained from all patients in order to participate in the study.

Participants will be recruited according to the following criteria:

Inclusion Criteria	Exclusion Criteria
1) Aged 18-40 with unilateral ACL injury 2) Sporting injury with a Tegner score of 7 3) Pre-op serum vitamin D level <20 ng/ml 4) 4 months post-ACLR with serum Vitamin D level remained <20ng/ml 5) LSI for quadriceps strength <70% of contralateral leg at 4-month isokinetic assessment 6) Both knees without history of injury/prior surgery	1) Concomitant bone fracture, major meniscus injury or full-thickness chondral injuries requiring altered rehabilitation program post-operatively 2) Pre-operative radiographic signs of arthritis 3) Metal implants that would cause interference on MRI 4) Non-HS graft for ACLR 5) Patient non-compliant to the rehabilitation program 6) Regular sunbed users

Method

<u>Intervention</u>

For the proposed study, we will use 2000IU/day as advised by the Endocrine Society (4). Previous study has shown 2000 IU/day for 4 months showed significant improvement of muscle strength (5). Therefore, for the proposed study, we will use 2000 IU/day (a standard formulary used in Hospital Authority in our cluster), for a duration of 16 weeks.
Subjects will be randomized into 2 study groups:
Group 1: Placebo group
Group 2: Intervention group - patients receive daily dose of 2000 IU of vitamin D3 supplements

All study tablets including the placebo will be manufactured according to Good Manufacturing Practice (GMP) guidelines for quality assurance. They will be taken with water at breakfast, 2 tablets at a time and once daily. The treatment will last for 16 weeks after which the supplementation will be stopped. Subjects will continue with their usual lifestyles in diet and physical activity without receiving any other treatment for muscle health. The procurement of study tablets will be covered by departmental funding.

Outcome Measurements

1. Anthropometric Measurement
   Body Mass Index (BMI) would be calculated by the measured height and weight at 4 months post ACLR before vitamin D supplements, during the eighth week of vitamin D supplements, after the completion of 16 weeks vitamin D supplements, and 12-months post-operation. We would measure the waist circumference as well. Subcutaneous fat would be measured using skinfold techniques for the triceps brachii and biceps brachii.
2. Biochemical Assays
   Blood samples will be taken under non-fasting conditions. Serum / plasma obtained will be immediately stored at -80°C until analysis. Serum 25(OH) Vit-D assay: Serum 25(OH)Vit-D levels will be measured by commercial 25(OH) Vitamin D ELISA kit (Abcam ab213966) according to the manufacturer's instruction, providing the quantitative determination of 25(OH) Vitamin D3 and 25(OH) Vitamin D2. Sensitivity: 1.98 ng/ml (Range: 0.5 ng/ml - 1010 ng/ml).
3. Radiological Assessment
   (a) Magnetic Resonance Imaging (MRI)
   Muscle volumes of quadricep muscle are measured using a 1.5 or 3.0 Tesla MRI Scanner. Axial (3mm thick cut) T1W images are obtained from the anterior superior iliac supine (ASIS) to patella. Quadriceps muscles were manually outlined in each axial slice. Muscle volume was calculated by summing all of the slice-multiplied by slice thickness. The quality of the muscle is assessed by analyzing the fat content of the muscle mass using technique that has been reported by Reeder et al (6). Bilateral legs will be performed before the start of the vitamin supplementation (4 months post-op) and with the injured side repeated after the completion of the 16-weeks supplementation (8 months postop).The uninjured side will be used as reference for 'normal volume'.
4. Self-reported activity level and muscle function
   (a) Lysholm Score (7)
   Lysholm Knee Score is a questionnaire that examines knee-specific symptoms and function of daily living. It consists of eight items, with a total score ranging from 0 to 100 and a higher score indicates a better outcome with fewer symptoms of disability.
   (b) International Knee Documentation Committee Subjective Knee Form (IKDC) (8)
   IKDC is a self-reported questionnaire that measures symptoms, knee function and activity of daily living. The questionnaire consists of 10 questions, with a total score ranging from 0 to 100 and a higher score indicates greater knee function.
   (c) Tegner Score (9)
   The Tegner Activity Scale was used to assess activity level that related to sports on a scale of 0 to 10. Zero represents a low activity level and 10 represents the highest activity level.
   (d) Physical Activity Questionnaire (10)
   Level of physical activities during the past year will be evaluated with a validated Chinese version of the quantitative physical activity questionnaire adapted from Baecke et al.

5. Isokinetic muscle strength assessment
   The dynamometer (Biodex System 4, Biodex Medical Systems Inc., New York, USA) will be used. Subjects will perform a standardized warm-up exercise (5 min cycling) followed by the test. Concentric/concentric contractions of knee extension/flexion will be tested at 60°/s and 180°/s (11). Subjects will be seated on the dynamometer chair with their hips flexed to 85°.
6. Knee laxity
   (a) Passive knee laxity
   To measure the anterior-posterior knee laxity, the KT-1000 knee ligament arthrometer (MEDmetric Corp, San Diego, CA, USA) will be used. Manual force test will be applied until 30lb sound signal is activated. Three trials will be performed. A side difference of 3 mm or above is considered clinically relevant (12).
7. Biomechanics - Motion Analysis
   The kinematics will be assessed by the skin marker-based motion analysis system (Vicon MX, Oxford, UK) with the lower-body marker setup, following the OSTRC standard protocol using 16-camera and 16 reflective skin markers.
   (a) Single leg hop (SLH) task
   The SLH test will be performed as reported in the previous studies reported (13). Three trials will be performed on each leg followed by familiarization. The SLH will be deemed valid if the patients can achieve a maximal hop distance while maintaining their balance for at least 2s after landing.
   (b) Single leg squat (SLS) task
   The subject will start at the upright standing position with their toes facing forward and squat down at a self-determined speed. Once the pre-assigned flexion angle is reached, subjects will be instructed to hold the position for 10 second. A trial will be invalid if the subject cannot maintain the proper balance. All participants will practice sufficiently to achieve the prescribed knee angle (40°-50° is acceptable).

Sample Size Estimation and Power Calculation

For objective 1, when the sample size is 92, a bivariate correlation test will have 90% power to detect at the 0.050 level with a correlation coefficient of 0.3. The proposed sample size (150) will be sufficient to detect the difference and enables the fulfillment of objective 2 and 3 in this study (with an estimated 40% quadriceps strength deficit at 4-month post-op). For objective 2, when the sample size in each group is 30, a repeated-measure ANOVA (with three repeated measures) will have 90% power to detect at the 0.050 level, with an effect size of 0.35. (G*Power 3.1.9.4).

Data Processing and Analysis

Data will be reported as the mean ± standard error of the mean, except for non-normally distributed data which are reported as the median, minimum and maximum. All Statistical analysis will be performed using the Statistical Package for Social Sciences (SPSS) 25. Statistical significance will be set at $p < 0.05$. For objective 1, a bivariate Pearson's correlation will be used to detect correlation between serum vitamin D levels and muscle strength and size at 4-month post-op, controlled for anthropometric variables. For objective 2, data will be analyzed by a repeated-measure ANCOVA (Control vs vitD supplementation; with repeated measures at 4-, 8-, 12-month post-op; other anthropometric variables as covariance).Primary outcome would be quadriceps muscle strength. These statistical tests will be performed after checking the normal distribution with Kolmogorov-Smirnov test, otherwise, a non-parametric

equivalent will be used. The “Intention-to-Treat” principle will be followed for data analysis purposes on those who have completed 16-week treatment. “Protocol Evaluable Analysis” will also be conducted using the 80% compliance as the cut-off. This trial will be registered at a clinical trial registry (ClinicalTrials.gov) before the study is implemented.

Facilities and Major Equipment

The proposed study will be conducted in the Prince of Wales Hospital (PWH). Subject inclusions, follow-up visits, clinical examinations, and questionnaire fillings will be conducted in the Out-patient Clinic of the Department of Orthopaedics and Traumatology, which is located at the 1/F of Li Ka Shing Specialist Clinic (North Wing) in the hospital. Biodex isokinetic dynamometer (Biodex System 4, Biodex Medical Systems Inc.), owned by our department, is available in Physiotherapy Department at G/F of PWH. Processing of blood samples for serum Vitamin D levels will be conducted in the Li Ka Shing Institute of Health Sciences (LiHS) in PWH, in which a full gear of centralized research facilities is available, including standard equipment for biochemical assays and molecular biology, such as large capacity refrigerated centrifuges in Centrifuge Laboratory (room 1009). 25(OH) Vitamin D Elisa Kit (ab213966) will be used for the analysis of the Vitamin D levels. Body composition including, lean body mass and body fat, as measured by bioelectrical impedance analysis (BIA, InBody Technology, US), which is owned by our department. (http://www.lihs.cuhk.edu.hk/enus/home.aspx). MRI scans will be performed at iRad Medical Diagnostic Centre with Philips Ingenia 1.5T MRI machine.

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