Research subjects

This study will recruit 18 female patients with mild to moderate unilateral knee OA. Multiple sources are utilised in recruiting the patients, including direct mailings, advertisements in popular newspapers, and several community approaches. Participants will be eligible for recruitment if they fulfilled the following inclusion criteria: (1) Currently experiencing objective functional limitations (2) aged between 40 and 70 years old, (3) symptomatic unilateral knee OA and (4) not participating in any regular resistance training.

A two-stage protocol will be employed in defining patients with knee OA: evidence of osteophytes based on radiographic imaging and either a grade 2 or 3 of the target knee upon bilateral standing anterior-posterior radiograph. A certified radiologist or physiatrist performed the reading of the X-rays. Meanwhile, potential participants will be excluded from this study if (1) their health status contradicted the use of a tourniquet, (2) currently suffering from peripheral vascular disorders or any condition contradicting subjecting them to exercise training, and (3) high blood pressure defined by a diastolic blood pressure > 100 mm Hg or resting systolic blood pressure > 160 or < 100 mmHg. Overall, patients with a history of other health conditions precluding safe participation will be excluded. The experiments will be performed upon receiving the written and oral consent of each participant.

Experimental design

This study is a single-blind cross-over experimental design. The participants will be trained to practice the motor movements before the formal test to enhance the test accuracy. Each participant have to perform the 1-repetition maximum (1-RM) test of the unaffected limb prior to the formal exercise, which is then followed by randomly conducting three resistance exercise tests by drawing lots: 1. A 30% 1-RM resistance exercise with BFR of 70% arterial occlusive pressure (AOP) (BFR group); 2. A 70% 1-RM resistance exercise without BFR (RES group); 3. A 30% 1-RM

resistance exercise without BFR (CON group). The ishout period of each test is 72 hours. In order to avoid hormonal effects, the rhythmic test is performed in the afternoon (14:00 to 16:30). The blood collection site is the elbow vein and the blood samples are assessed for systemic biochemical markers. Specifically, blood sampling is conducted before and immediately after exercise, and subsequently at 15 min and 30 min post-exercise.

Maximum strength test

The exercise prescription guidelines proposed by the American College of Sports Medicine (ACSM) are followed in executing the participants' 1-RM assessments. Leg curl of the normal limbs and leg extension are the main contents of the test. The participants had to familiarise themselves with the test procedure before executing a warm-up exercise, comprising numerous knee resistance exercises. The participants chose the initial weight (50%-70% 1-RM) during the test in line with the range of self-predictive ability. Upon completing each test, the load is amplified by 10% to 20% until the participant could not execute the programmed frequency of repetitions. The participants maintained the same joint range of motion and speed. Resultantly, a 1-RM is achieved within four tests with a corresponding resting time of three min between each test. The 1-RM value is documented as the weight of the last executed test.

Exercise protocols

The exercise load of 30% 1-RM is utilised by the CON and BFR groups, whereas the exercise load of 70% 1-RM is used by the RES group. The healthy limbs in each test required six sets of knee extension and flexion exercises utilising a sitting posture bending exerciser (HS-SLC, Life Fitness, Schiller Park, IL, USA) and a sitting leg extension exerciser (HS-LE, Life Fitness, Schiller Park, IL, USA). Both devices used similar movement modes that required the back of the body to be adjacent to the seat, holding the handles with both hands, and modifying the calf's force point to the ankle joint. The eccentric contraction and concentric contraction are three sec each, each set is repeated 15 times, with one min interval

between each set. For the BFR group, the participants are required to wear a pneumatic cuff with 70% AOP on their unaffected limbs (SC12L, Hokanson, Bellevue, WA, USA). This device assumed a straight cuff with a 12 x 124 cm in sized nylon sleeve on the outside. The cuff is worn on the upper third of the thigh (proximal end of the legs) during the exercise. Specifically, the cuff is adjacent to the lower limbs and perpendicular to the limbs when fastened. The device is detached upon completing the exercise.

Blood flow pressure setting

Following the test method described in a previous study, the participants' blood flow occlusion pressures are evaluated with a BFR cuff and a Doppler probe (DV-600, Marted, Ribeirao Preto, Sao Paulo, Brazil)[13]. While maintaining a supine position, the cuff is positioned on the proximal end of the participants' thighs and inflated gradually (i.e., 1 mmHg) until pressure is attained in which no arterial pulse could be further detected. The arterial occlusion pressure is recorded as the minimum pressure in which no pulse is detected. The average value is taken upon testing the left and right legs three times.

Perceived exertion

The rating of perceived exertion (RPE) scale is used to evaluate subjective load before and after exercise. Participants are informed that a rating of 6 depicts a rating of 6 meant they sensed no exertion, whereas 20 reflects they are exerting maximum effort and unable to further exert themselves[14].

Blood sample

Blood sampling is performed at various time points as described in the study design. The serum samples are allowed to stand for 30 min, centrifuged at 3000 revolutions per min (rpm) for 15 min, and stored at -80° C before usage. Serum IGF-1, GH, blood lactate (BLA), and testosterone are analysed using the double-antibody sandwich enzyme-linked immunosorbent assay (Enzyme-linked immunosorbent assay, ELISA).

Statistical analysis

Statistical analyses are performed using the SPSS 24.0 software (IBM, USA). Data

normality is assessed using the Shapiro-Wilk normality test. All the data conformed to normality assumptions and they are presented as mean ± standard deviation (Mean ± SD). Alterations in testosterone, BLA, IGF-1, and GH levels at different periods are tested using a two-way repeated-measures analysis of variance (ANOVA). The same statistical test is employed in analysing the alterations of RPE at pre-and post-exercise. All the analyses for each group are checked for the main and interaction effects. If either of the two effects is significant, the multiple comparisons are conducted using the Newman-Keuls method. Statistical differences are considered at P-values less than 0.05.