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ORIGINAL REPORT

EFFECTS OF ISOKINETIC KNEE MUSCLE TRAINING ON BONE MORPHOGENETIC PROTEINS AND INFLAMMATORY BIOMARKERS IN POST-TRAUMATIC OSTEOARTHRITIS AFTER ANTERIOR CRUCIATE LIGAMENT INJURY: A RANDOMIZED TRIAL

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Objective: **To determine the effects of isokinetic training of the knee muscles on bone morphogenetic proteins and inflammatory biomarkers in post-traumatic osteoarthritis after anterior cruciate ligament injury in university football players.**

Methods: **A total of 60 participants with post-traumatic osteoarthritis after anterior cruciate ligament injury were randomly allocated into 3 groups: isokinetic training ($n = 20$), sensory motor training ($n = 20$) and control ($n = 20$) groups. The groups underwent different training programmes for 4 weeks. Clinical and biochemical values were measured at baseline, 4-week, 8-week and 6-month follow-ups.**

Results: **At the end of the 4 week training period the isokinetic group showed more significant changes in pain intensity and functional disability than the sensory motor training or control groups ($p < 0.001$). There was no significant changes in bone morphogenic protein measures, (e.g. bone morphogenic proteins 2, 4, 6, and 7) in any of the groups. There was positive changes in inflammatory markers (CRP, TNF-α, IL-2, IL-4, IL-6) in the isokinetic training group compared with the other 2 groups ($p < 0.001$).**

Conclusion: **Isokinetic training results in greater improvements in pain and functional disability than sensory motor training in post-traumatic osteoarthritis after anterior cruciate ligament injury in university football players. The isokinetic training programme had a beneficial effect on levels of inflammatory biomarkers and negligible effect on bone morphogenic proteins.**

Key words: isokinetic training; pain intensity; functional disability; bone morphogenic protein; inflammatory biomarker; post-traumatic osteoarthritis.

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LAY ABSTRACT

Recent research has shown that conventional training in footboll players with post-traumatic osteoarthritis improves pain, functional activity, cartilage morphology and inflammatory reactions. However, little is known about the effect and mechanism of isokinetic training on bone morphogenic proteins (BMP) (BMP 2, 4, 6, and 7) and inflammatory markers ((C-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), and interleukins (IL)-2, IL-4, and IL-6)) in post-traumatic osteoarthritis after anterior cruciate ligament injury. Comprehensive understanding of the effects of isokinetic training on the relationship between biomechanical and biochemical changes would promote this clinical condition in a positive way. Hence, these type of training protocols would modify the risk and reduce the negitive consequences of anterior cruciate ligament injury.

Post-traumatic osteoarthritis (PTOA) of the knee is a degenerative joint condition, often resulting from occupational or sports injuries, which may be caused by excessive or abnormal loads on the joint. Approximately 12% of soft-tissue injuries to the knee lead to anterior cruciate ligament (ACL) rupture (1, 2). Elite football players are highly prone to ACL injury, caused by twisting movements at the knee and an imbalance in the strength of the hamstring and quadriceps muscle (3). The incidence of ACL injury in football (soccer) in USA is 18.8%, which is higher than in other field games, such as American football, beach soccer and futsal (4). The risk factors commonly associated with ACL injuries are; decreased muscle strength; torque ratio (hamstring/quadriceps; H/Q); and muscle weakness (5). Imbalance in H/Q peak torque strength also affects the players' posture and physical activities (6).

Recent reports have shown that football players with ACL injuries have a high probability of developing PTOA, irrespective of the treatment provided. PTOA gradually affects the joint capsule and articular cartilage, and subsequently affects the subchondral bone. Lack of training and poor body posture during football matches are the root cause of these injuries (7). The risk of developing PTOA puts extreme pressure on players and healthcare personnel to understand and identify the mechanism of deterioration after ACL injury. Arthrogenic muscle inhibition (AMI)

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in the quadriceps muscle following ACL injury is a potential risk factor for knee PTOA (8). Several other factors, such as abnormal shock attenuation, load distribution and biomechanical forces, also play a considerable role in development of knee PTOA (9). Subtle rupture of the ACL may injure the cartilage and trigger metabolic (bone morphogenic protein; BMP) and inflammatory changes in the joint and bone (10). Inflammatory changes, such as low-grade cellular infiltration, cytokine production, inflammatory activation of articular chondrocytes, synoviocytes and other joint tissue cells are also common in PTOA (11).

Surgical intervention and a follow-up rehabilitation programme result in a positive prognosis in most cases of ACL injury (12, 13). However, H/Q balance is not achieved and the imbalance may exist for years after injury and surgery (12). Recent studies show that therapeutic and physical training in players with PTOA improves pain, functional activity, cartilage morphology and inflammatory reactions (13). Compared with other types of muscle training, isokinetic training of the quadriceps and hamstring muscles results in significantly better improvements in joint fluid composition, fluid viscosity and molecular weight of hyaluronan (14). Weak Q/H torque ratio may lead to reduced knee function through higher rates of distribution of loads across the joint. Thus maintaining the balance of the hamstring and quadriceps is vital; this can be achieved through isokinetic training (14). However, the effect and mechanism of isokinetic training on BMPs (e.g. BMP 2, 4, 6, and 7) and inflammatory markers (CRP, TNF-α, IL-2, IL-4, IL-6) in PTOA after ACL injury are unclear.

Moreover the role and interaction of BMPs and inflammatory markers in PTOA are unknown. Comprehensive understanding of the effects of isokinetic training on the relationship between biomechanical and biochemical changes would promote this clinical condition in a positive way. Hence, these type of training protocols would modify the risk and reduce the negitive consequences of ACL injury.

The primary objective of this study was to determine the effects of isokinetic training of the knee muscles on BMPs and inflammatory biomarkers in post-traumatic osteoarthritis after ACL injury in university football players. It was hypothesized that isokinetic knee muscle training would have effect on these factors. This information may help football players to decrease the risk of PTOA or to minimize the rate of progression of this condition.

MATERIAL AND METHODS

Study design

The study was a double-blinded randomized control study. A total of 60 university football players with PTOA after ACL injury were randomized and allocated equally into 3 groups in a 1:1:1 ratio according to a 3-block randomization method: isokinetic training (IKT-G) ($n=20$), sensory motor training (SMT-G) ($n=20$) and control (Control-G) ($n=20$) groups. The study was approved by the departmental scientific ethics committee (study number RHPT/019/008) and was conducted according to the principles of the Declaration of Helsinki 1964 and Declaration of Tokyo 1975. The study was performed and presented in accordance with Consolidated Standards of Reporting Trials (CONSORT) guidelines.

The study was conducted in the Department of Physical Therapy and Health Rehabilitation, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia. Participants were recruited from the University Hospital and King Khalid Hospital, Al-Kharj, Saudi Arabia. A sports therapist at the department evaluated the participants according to the eligibility criteria. Clinical trial registration number: CTRI/2019/04/018593.

Participants

In the initial phase, all participants were provided with a study information form with information on the research questions, study design, intervention procedures, outcome measures, study duration, and harms and benefits of the research. Subjects who read the information and consented to participate underwent primary screening for final selection. Inclusion criteria for selection of the subjects were: university football players; age group 18–25 years; male players; chronic (≥3 months) OA after ACL injury; and 4–8 pain intensity on the visual analogue scale (VAS). Exclusion criteria were: severe musculoskeletal, neural, somatic, or psychiatric conditions; waiting for surgery; alcohol or drug abuse; or involvement in other weight and balance training programmes. Subjects with other soft-tissue injuries, fracture of the lower limbs and pelvic bone, or deformities were also excluded.

Interventions

All 3 groups followed a 4-week rehabilitation protocol, supervised by a physiotherapist with 5 years' experience. The protocol specifically emphasized balance training. Subjects were also instructed and advised to exercise at home.

Isokinetic training group. Prior to isokinetic training, subjects in the IKT group ($n=20$) were instructed to perform a 5-min warm-up, followed by slow stretching of the hamstring and quadriceps muscles. They were instructed to sit in an isokinetic dynamometer with their hips flexed at 90°. Velcro® fixation straps were tied around the chest, hip and the distal thigh of the training limb to prevent unnecessary movements. The training knee was kept at a 90° flexed position, and the dynamometer axis was aligned with the centre of the lateral femoral condyle. The lever arm was customized according to the subject's leg length, and resistance was applied anterior to the ankle joint.

To reduce the risk of participants the required modifications and procedures were performed according to the user's manual. The knee was tested from 0° to 120° of flexion, where 0° was considered full extension.

Subjects were familiarized with the exercise by showing them video clips of a model, then allowing them practice attempts. Once they had mastered the exercise they were instructed to perform it at an angular speed of 60, 90 and 120°/s with 15 repetitions in 3 sets. Rest periods of 30 s between each set, and 60 s between each speed, were given. Training was performed on 5 days a week for 4 weeks. Subjects were monitored and instructed by a supervisor throughout the training. Outcome parameters were assessed by a different examiner, who was experienced in handling isokinetic devices (15).

Sensory motor training group. In the SMT group, subjects ($n=20$) were trained in sensory motor training exercises specific to the knee joint. The training was given in 3 stages; static, dynamic and functional. All exercises were performed 5 times in 1 set, for 3 sets, with a sufficient with 5 minutes rest period between sets. The exercise protocol was designed so that the level of difficulty increased. The subjects were not progressed to the next level of difficulty until they had completed the previous level. In the static phase subjects were instructed to stand in an erect position for 30 s on a firm surface and 30 s on a soft surface. They were then instructed to stand on one leg (the affected limb) with eyes closed for 10 s on a firm surface and 10 s on a soft surface, followed by a half knee-bend position for 10 s. In the dynamic phase they were instructed to perform a forward stepping thrust for 30 s and T-band kick exercise for 30 s. The functional phase began with toe skipping for 20 m (straight, inward and outward rotation) and heel skipping for 20 m (straight, inward and outward rotation). Subjects were then instructed to perform regular squats (10 times) bilaterally and unilaterally with the support of a wall and away from the wall. Once trained in the above exercises they were instructed to perform the balance exercises on a wobble board (16).

Control group. In the control group (Control-G) the subjects ($n=20$) were given instructions about the type of exercise, and the procedure for stretching and strengthening the specific muscles to be performed at home. Initially the exercises were demonstrated by the therapist, and the subjects practiced in the presence of the therapist and clarifications were given. These home-based exercises were printed in a hand manual with easy-to-follow images and language. The first part of the manual contained the do's and don'ts for the study period. The next part of the manual contained different stretching and strengthening exercises for quadriceps, hamstrings, glutei and calf muscles. Subjects performed these exercises 10–15 repetitions/day, 5 days a week, for 4 weeks. Stretching was focused on each muscle group for 3 repetitions of 15 s per muscle group (17).

Outcome variables

Pain intensity. Pain intensity was measured on a visual analogue scale (VAS), which comprised a 10-cm horizontal line with one end representing "no pain at all" and the other end pain "pain as bad as it could possible be." Each subject was instructed to indicate their pain perception score on the line. The reliability and validity of the VAS in application of musculoskeletal conditions was good (18).

Functional disability. Functional disability was measured with the Arabic version of the Western Ontario and McMaster Universities Arthritis Index (WOMAC) scale, which comprised 24 items divided into 3 subscales, such as pain, stiffness and physical function. The items were scored on a 0–4 Likert scale, on which 0 = none, 1 = mild, 2 = moderate, 3 = severe and 4 = extreme. Each subject was instructed to enter their perceived disability on the line. The lowest score was considered as no disability and highest score as maximum disability. The reliability and validity of the WOMAC scale in the application of knee osteoarthritis was good (19).

Bone morphogenic proteins. Blood samples were collected by a laboratory technician between 08:00h and 10:00h to avoid circadian issues. The procedure followed a standard protocol. After centrifugation the collected plasma samples were stored at –70º C. The required bone BMPs (BMP 2, 4, 6, and 7) in the concentrated plasma samples were analysed with an enzyme-linked immunosorbent assay (ELISA) (BMP ELISA Kit, Elab Science, Wuhan Elab Science Biotechnology Co. Ltd, China), which was used to measure the BMP level at various intervals (10).

Inflammatory biomarker. Blood samples (10 ml) were taken in sterile tubes between 08:00h and 10:00h. Serum was separated and centrifuged, then frozen at –70º C and stored. Serum levels of CRP, TNF-α, IL-2, IL-4, and IL-6 were measured by ELISA (My Bio Source, Multiplex Human Cytokine ELISA Kit, California, CA, USA), according to the manufacturer's guidelines. The lower and upper limits of detection were computed for each assay, and the mean percentages inflammatory biomarker values of samples were reported for statistical analysis (20).

Sample size

The required sample size was calculated using a previous pilot study with primary outcome data for pain intensity and an assumed power of 80% using 2-tailed test with significance level of 0.05. To detect the minimum effect size of 1.2 with mean difference of 2.0 and standard deviation (SD) of 0.5, the required sample size was 18 in each group. When considering 10% drop-out, the required samples size for each group was 20.

Randomization

An individual who was not involved in the data collection applied the randomization. The subjects were randomized to 3 groups (IKT-G, SMT-G, and Control-G) following a 3-block randomization method with 1:1:1 ratio. All prospective subjects who fulfilled the eligibility criteria were included in the study.

Blinding

Due to the study design it was not possible to blind the treating therapist. The subject and the therapist who was assessing the outcomes at baseline, after 4 weeks, 8 weeks and 6 months were blinded. Therefore, the treating and assessing therapists were different persons and the assessing therapist was blinded to the subject's treatment group. Subjects were instructed not to disclose the study procedures or treatment protocol to fellow subjects or to the assessing therapist.

Statistical analysis

Subjects' demographic characteristics were assessed using the Kolmogorov–Smirnov test to determine study homogeneity. Outcome data were presented as mean and SD, and repeated measures of ANOVA was performed to determine significant differences within the groups. One-way ANOVA was used for comparison

between groups and the statistical significance level was set at $p<0.05$. Tukey's *post hoc* pairwise comparisons between groups were performed in case of an overall statistically significant effect across the 3 groups. SPSS software (version 20.0) (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Participants

A total of 80 subjects were screened for the study, 60 were selected and randomized to 3 groups of 20: IKT-G, SMT-G and Control-G.

Five participants with a high level of pain (≥8 on the VAS), 6 participants with other musculoskeletal and joint injuries, 3 awaiting surgery, and 6 who declined to participate in the study were excluded (Fig. 1).

The intention-to-treat analysis method was considered in this study, but 2 participants from each group did not complete the study and were not included in the analysis. Six participants dropped out for personal reasons. Descriptive demographic analysis of subjects' characteristics (age, height, weight and BMI) were performed in all 3 groups at baseline and presented as mean and SD. Clinical parameters (VO_{2peak}, heart rate, years of playing and duration of injury) were measured to determine eligibility to participate in the exercise training programme. There was no difference between the groups in the demographic and clinical parameters at baseline (Table I).

Table I. Demographic details of the isokinetic training (IKT-G), sensory motor training (SMT-G) and control (Control-G) groups

Variable	IKT-G Mean (SD)	SMT-G Mean (SD)	Control-G Mean (SD)	*p*-value
Age, years	22.3 (1.2)	22.4 (1.5)	22.9 (1.7)	0.573*
Height, m	1.72 (0.15)	1.74 (0.14)	1.72 (0.15)	0.928*
Weight, kg	63.2 (1.6)	64.5 (1.6)	63.6 (1.4)	0.120*
BMI, kg/m^2	22.5 (1.5)	22.8 (1.3)	22.6 (1.4)	0.867*
VO_{2peak}, ml/kg/min	36.3 (3.4)	37.4 (4.2)	37.3 (4.2)	0.754*
HR, beats/min	168 (6.3)	169 (6.3)	168 (6.5)	0.906*
Years of playing, years	5.5 (1.5)	5.8 (1.5)	5.6 (1.7)	0.892*
Duration of injury, months	5.3 (0.2)	5.4 (0.6)	5.4 (0.3)	0.784*

*Non-significant.
BMI: body mass index; HR: heart rate; SD: standard deviation. IKT-G: Isokinetic training group, SMT-G: Sensory motor training group; VO: Oxygen volume.

Pain and functional disability

Inter-group analysis between the 3 groups at 4-week, 8-week and 6-month follow-ups revealed significance differences ($p<0.001$) after 4 weeks of training. Moreover, intra-group analysis of the 3 groups revealed significance differences ($p<0.001$); hence, all groups had improved considerably. Tukey's *post hoc* pairwise

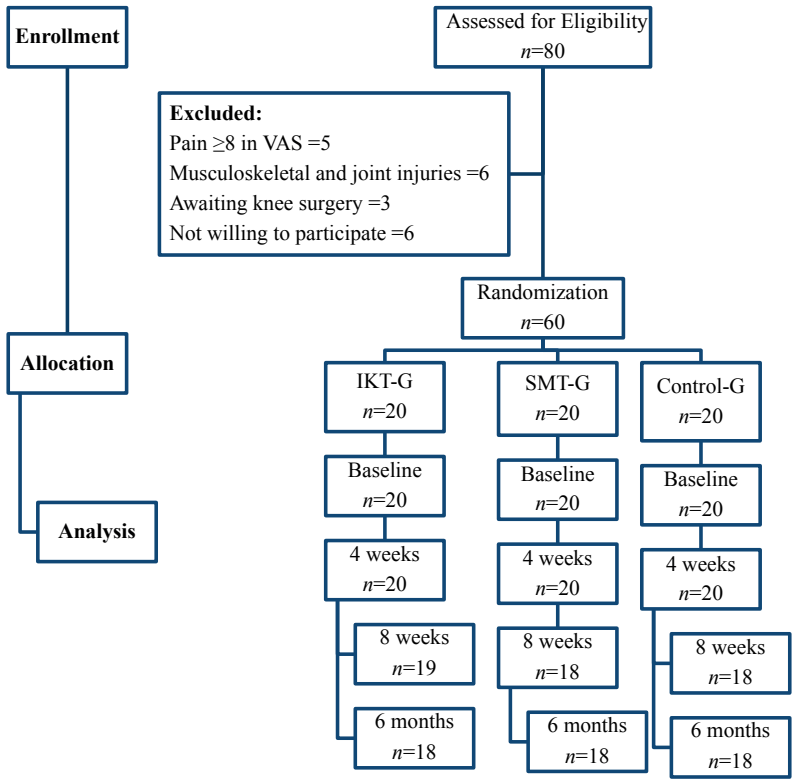

Fig. 1. Study flow chart. VAS: visual analogue scale; IKT-G: isokinetic training group; SMT-G: sensory motor training group; control-G: control group.

Table II. Visual analogue scale (VAS) score and Western Ontario and McMaster Universities Arthritis Index (WOMAC) analysis pre- and post-intervention in the isokinetic training (IKT-G), sensory motor training (SMT-G) and control (Control-G) groups

Variable	IKT-G	SMT-G	Control-G	*p*-value
Pain intensity (VAS)				
Baseline, mean (SD)	7.5 (0.4)	7.7 (0.6)	7.5 (0.5)	0.542*
4 weeks, mean (SD)	3.8 (0.2)	5.6 (0.3)	6.8 (0.4)	0.000**
8 weeks, mean (SD)	1.8 (0.4)	3.6 (0.3)	3.8 (0.4)	0.000**
6 months, mean (SD)	0.8 (0.3)	2.9 (0.2)	3.1 (0.2)	0.000**
Improvement, %	89	62	59	
p-value	0.000**	0.000**	0.000**	
Functional disability (WOMAC)				
Baseline, mean (SD)	71.85 (3.6)	71.02 (3.8)	72.62 (3.9)	0.590*
4 weeks, mean (SD)	38.32 (3.3)	58.54 (3.5)	65.43 (3.3)	0.000**
8 weeks, mean (SD)	24.32 (3.4)	38.74 (3.6)	45.43 (3.3)	0.000**
6 months, mean (SD)	13.07 (2.8)	26.66 (2.2)	39.09 (2.9)	0.000**
Improvement, %	82	62	46	
p-value	0.000**	0.000**	0.000**	

*Non-significant; **significant.
SD: standard deviation.

comparison analysis and the graphical representation between the groups reported that IKT-G had greater reduction in pain and functional disability than did the SMT-G and Control-G (Table II, Fig. 2).

Bone morphogenic proteins

BMP levels (BMP 2, 4, 6 and 7) were measured before and after 4 weeks of training in all the 3 groups. Follow-up measurements (after 8 weeks and 6 months) were also determined to study the short-term and intermediate effects of these training. There was no difference in BMP levels between the groups at 4-week, 8-week or 6-month follow-ups ($p>0.05$); hence the isokinetic training had a limited effect on BMP levels (Table III, Fig. 3).

Table III. Bone morphogenic protein (BMP) analysis pre- and post-intervention for the isokinetic training (IKT-G), sensory motor training (SMT-G) and control (Control-G) groups

Variable	IKT-G	SMT-G	Control-G	*p*-value
BMP-2, pg/ml				
Baseline, mean (SD)	658.36 (8.2)	656.12 (9.9)	655.38 (8.8)	0.702*
4 weeks, mean (SD)	655.88 (7.2)	657.92 (8.5)	654.28 (9.3)	0.571*
8 weeks, mean (SD)	656.78 (8.2)	656.92 (8.9)	655.48 (9.2)	0.794*
6 months, mean (SD)	655.98 (8.5)	657.83 (8.4)	655.28 (9.8)	0.987*
p-value	0.865*	0.955*	0.210*	
BMP-4, pg/ml				
Baseline, mean (SD)	642.45 (8.1)	643.89 (7.9)	642.12 (8.2)	0.850*
4 weeks, mean (SD)	643.35 (7.8)	642.98 (8.1)	641.28 (8.4)	0.801*
8 weeks, mean (SD)	642.41 (8.2)	642.85 (8.2)	643.19 (8.4)	0.973*
6 months, mean (SD)	642.46 (8.6)	642.81 (7.6)	644.15 (7.9)	0.863*
p-value	0.990*	0.985*	0.841*	
BMP-6, pg/ml				
Baseline, mean (SD)	699.12 (9.5)	698.92 (9.3)	699.19 (8.9)	0.997*
4 weeks, mean (SD)	698.26 (8.9)	699.91 (8.8)	698.09 (8.8)	0.856*
8 weeks, mean (SD)	698.22 (9.3)	698.95 (8.9)	698.08 (8.6)	0.967*
6 months, mean (SD)	698.35 (8.5)	699.54 (8.3)	698.01 (8.5)	0.897*
p-value	0.990*	0.990*	0.986*	
BMP-7, pg/ml				
Baseline, mean (SD)	240.76 (8.2)	239.53 (7.3)	241.62 (8.2)	0.810*
4 weeks, mean (SD)	241.69 (7.2)	240.33 (6.3)	240.81 (7.5)	0.890*
8 weeks, mean (SD)	241.86 (8.8)	240.02 (6.5)	239.55 (7.2)	0.733*
6 months, mean (SD)	240.69 (7.2)	239.67 (8.3)	240.82 (7.9)	0.925*
p-value	0.974*	0.992*	0.930*	

*Non-significant.
SD: standard deviation.

Inflammatory biomarkers

Inflammatory biomarkers (CRP, TNF-α, IL-2, IL-4 and IL-6) were measured at baseline, 4-week, 8-week and 6-month follow-ups. There were no significant differences in CRP level ($p>0.05$) between groups at the 4-week follow-up. However, there were significant differences between groups for all inflammatory biomarkers ($p<0.001$) at 8-week and 6-month follow-ups (Table IV). Tukey's *post hoc* pairwise comparison

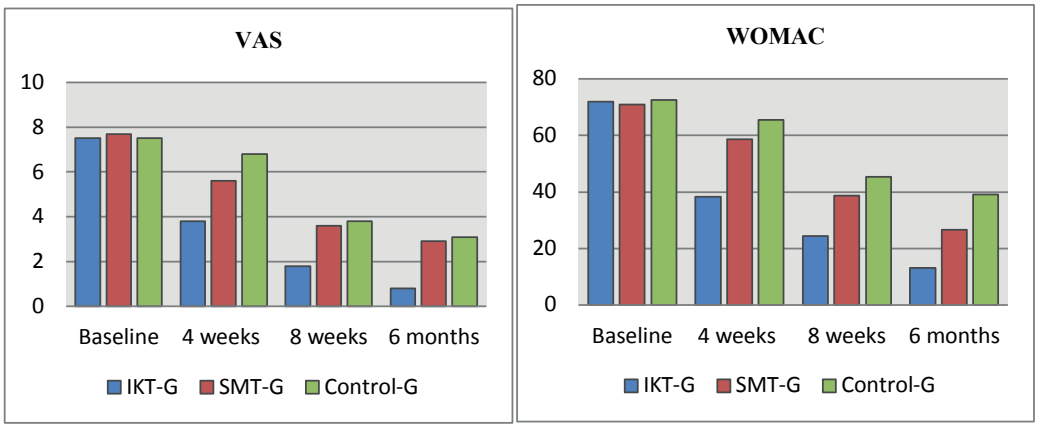

Fig. 2. Mean visual analogue scale (VAS) and Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores in the isokinetic training (IKT-G), sensory motor training (SMT-G) and control (Control-G) groups at baseline and follow-up.

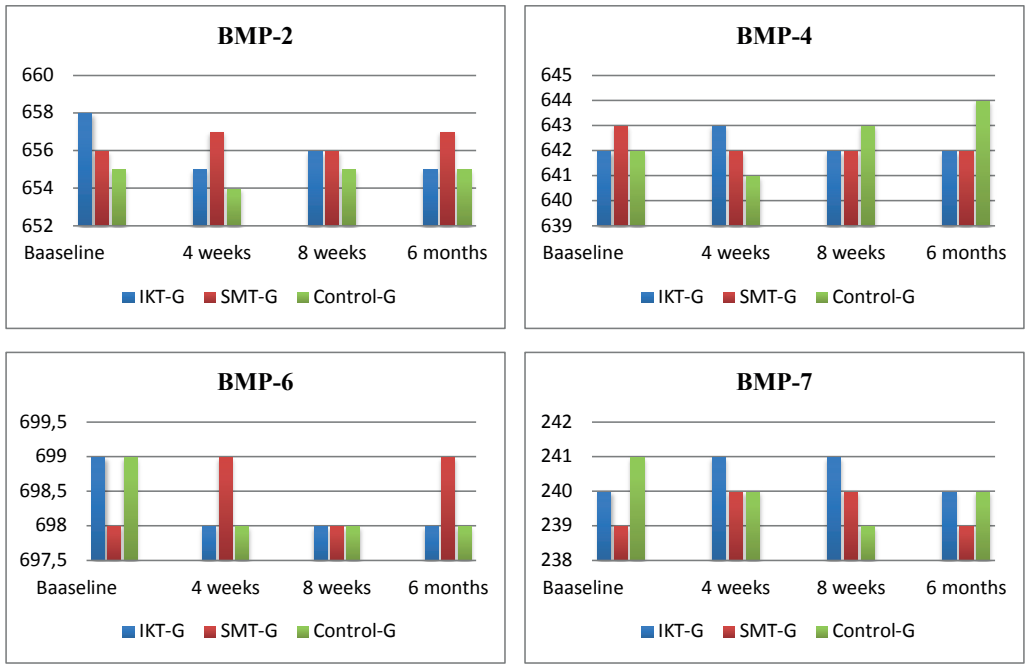

Fig. 3. Mean levels of bone morphogenic proteins (BMPs) BMP 2, 4, 6 and 7 in the isokinetic training (IKT-G), sensory motor training (SMT-G) and control (Control-G) groups at baseline and follow-up.

analysis and the graphical representation of the differences between the groups at 6 months shows a greater tendency to improvement in the isokinetic training group than in the sensory motor training and control groups (Fig. 4).

Table IV. Analysis of pro-inflammatory biomarker levels pre- and post-intervention for the isokinetic training (IKT-G), sensory motor training (SMT-G) and control (Control-G) groups

Variable	IKT-G	SMT-G	Control-G	*p*-value
CRP, mg/l				
Pre, mean (SD)	1.57 (0.5)	1.52 (0.4)	1.49 (0.5)	0.891*
4 weeks, mean (SD)	1.12 (0.5)	1.45 (0.5)	1.40 (0.4)	0.193*
8 weeks, mean (SD)	0.67 (0.4)	1.05 (0.4)	1.35 (0.5)	0.002**
Post, mean (SD)	0.39 (0.4)	0.79 (0.3)	1.28 (0.4)	0.000**
p-value	0.000**	0.000**	0.714*	
TNF-α, pg/ml				
Pre, mean (SD)	15.52 (0.6)	15.67 (0.6)	15.59 (0.5)	0.812*
4 weeks, mean (SD)	10.56 (0.5)	14.52 (0.4)	15.32 (0.4)	0.000**
8 weeks, mean (SD)	9.56 (0.4)	13.43 (0.5)	15.26 (0.6)	0.000**
Post, mean (SD)	7.5 (0.4)	12.41 (0.4)	15.25 (0.5)	0.000**
p-value	0.000**	0.000**	0.320*	
IL-2				
Pre, mean (SD)	12.44 (0.6)	12.58 (0.6)	12.32 (0.5)	0.539*
4 weeks, mean (SD)	13.68 (0.5)	12.43 (0.4)	12.46 (0.5)	0.000**
8 weeks, mean (SD)	14.61 (0.5)	13.34 (0.6)	12.51 (0.4)	0.000**
Post, mean (SD)	15.92 (0.4)	13.28 (0.5)	12.28 (0.4)	0.000**
p-value	0.000**	0.000**	0.552*	
IL-4				
Pre, mean (SD)	39.21 (0.5)	39.52 (0.7)	38.99 (0.6)	0.114*
4 weeks, mean (SD)	45.64 (0.4)	42.51 (0.4)	38.62 (0.5)	0.000**
8 weeks, mean (SD)	58.72 (0.4)	51.21 (0.6)	38.52 (0.6)	0.000**
Post, mean (SD)	64.21 (0.4)	55.28 (0.5)	38.38 (0.6)	0.000**
p-value	0.000**	0.000**	0.075*	
IL-6				
Pre, mean (SD)	5.9 (0.5)	5.5 (0.6)	5.4 (0.5)	0.067*
4 weeks, mean (SD)	3.5 (0.4)	5.2 (0.4)	5.3 (0.6)	0.000**
8 weeks, mean (SD)	2.7 (0.3)	4.5 (0.3)	5.3 (0.6)	0.000**
Post, mean (SD)	1.6 (0.5)	3.3 (0.4)	5.1 (0.5)	0.000**
p-value	0.000**	0.000**	0.603*	

*Non-significant; **significant. CRP: C-reactive protein; IL: interleukin; TNF: tumour necrosis factor; SD: standard deviation.

DISCUSSION

The main objective of this study was to determine the clinical, bone morphological and inflammatory effects of isokinetic training and sensory motor training in PTOA after ACL injury in university football players. Pain and functional disability were significantly improved in the isokinetic training group compared with the sensory motor training and control groups. However, all 3 groups showed little or no improvement in BMPs after exercise training. Analysis of inflammatory biomarkers revealed that the isokinetic training group improved more than the other groups. These positive changes in the levels of inflammatory cytokines may play a role in preventing further joint injuries.

Isokinetic training

Isokinetic training was applied at different angular velocities in this study (60, 90 and 120°/s and at high peak torque. Cheung et al. observed that training at different angular velocities and high peak torque improved knee muscle strength and H/Q ratio in football players (21). These biomechanical changes clinically reduce the level of pain in subjects with PTOA, and improve the functional capacity of the patients, as

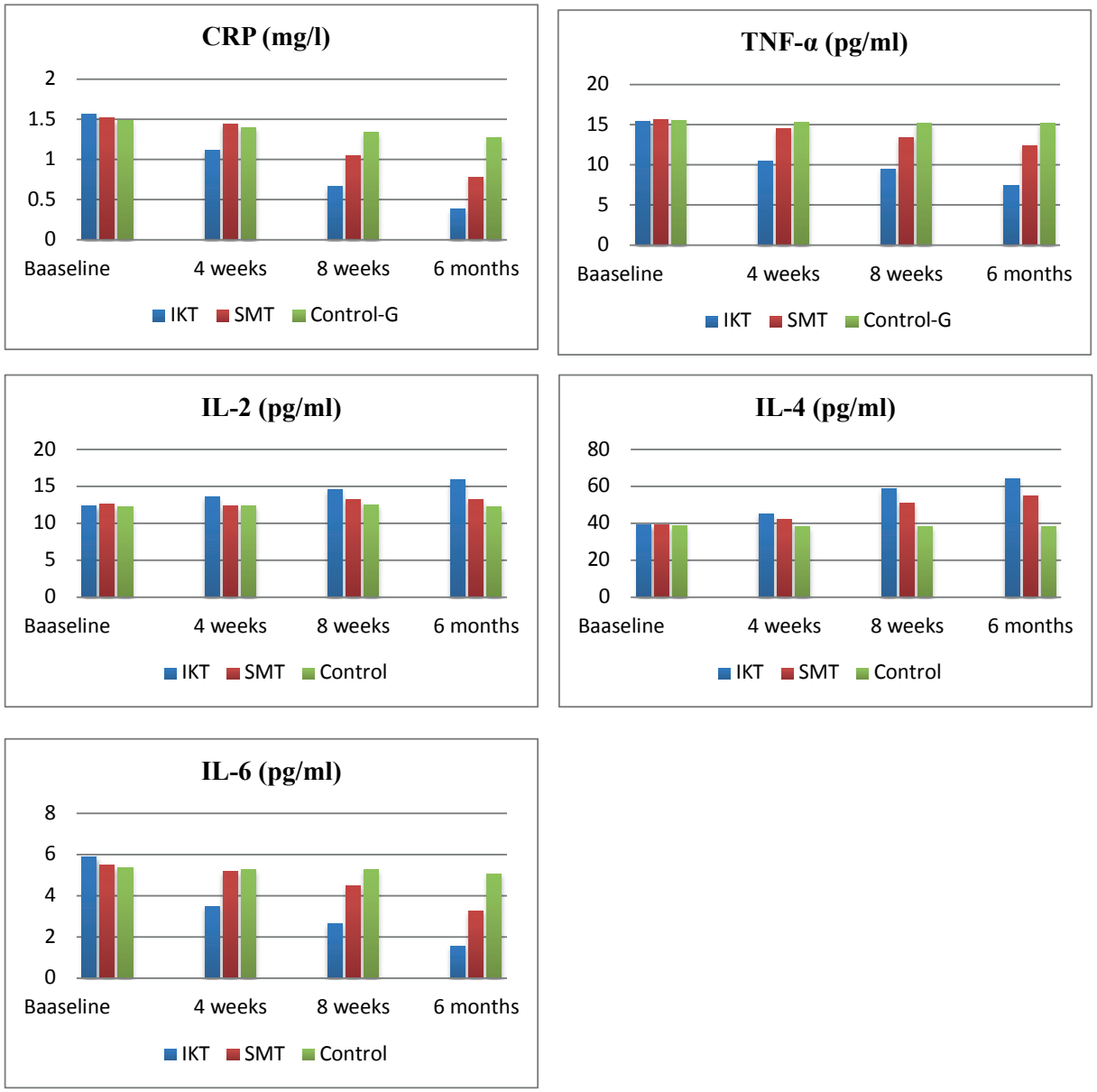

Fig. 4. Mean levels of C-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), interleukin (IL)-2, IL-4 and IL-6 in the isokinetic training (IKT), sensory motor training (SMT) and control (Control-G) groups at baseline and follow-up.

shown by Eniseler et al. (22) and Ardern et al. (23), who also described that improving H/Q ratios was key to preventing further injury. It was also shown inflammatory biomarker values that patients with OA with a lower H/Q ratio and low peak torque production benefit from an intense isokinetic training programme. In this type of training the concentric action of the quadriceps is controlled by the eccentric action of the hamstring, which maintains the dynamic stability of the knee joint (24, 25).

The current study found no or little change in BMPs after isokinetic training. The reason for this is unknown. Local expression of BMPs was not affected by any of the exercises. However, it was shown that isokinetic training had a positive impact on bone mineral density and bone mineral content, which is in accordance with Nickols-Richardson et al.'s research (26). Moreover, the current study found significant improvement in levels of pro-inflammatory biomarkers after isokinetic training in PTOA after ACL injury. There was a decrease in CRP, TNF-α, IL-6 levels and increase in IL-2 and IL-4 levels after isokinetic training. These changes may reduce the inflammatory process in PTOA, in agreement with the study of Helamark et al. (27). Current research suggests that the use of balanced controlled exercises in isokinetic

training further reduces the joint injury and improves the regeneration process, which has a positive correlation with the levels of inflammatory biomarkers (28).

Sensory motor training

The current study also found little improvement in pain and functional disability in the sensory motor training group compared with the control group. Sensory motor training exercises generally restored the muscle strength through specific exercises, which facilitated the action of joint receptors, which, in turn, enhanced the proprioception, joint stability and overall function of the joints. This reaction was in accordance with central integration theory (29), in which sensory motor training activates the reflective muscle action through various body positions and different bases of support. No change in BMPs was found in the sensory motor training and control groups. The current study showed that there was little improvement in inflammatory biomarker levels in PTOA after sensory motor training. These results are in agreement with those of Aguiar et al., who found the sensory motor training could be used to modify levels of pro-inflammatory cytokines, which helped to modify pain and improved function in subjects with PTOA (30). The current study also confirmed an association between inflammatory cytokine levels and pain intensity, in accordance with a study by Imamura et al. (31).

Study strengths and limitations

The main strengths of this study were the study method and the sample size, which was calculated from a previous pilot study; hence, the results can be generalized to the general population. Some limitations were observed during implementation of this study. First, this study did not measure isokinetic parameters, such as concentric, eccentric muscle strength evaluation and H/Q ratio. Future studies should measure these isokinetic parameters in PTOA. Secondly the associations between the clinical, BMPs and inflammatory biomarkers in PTOA after different training protocols were not analysed. Finally, the long-term effects of different training protocols (after 1 year) were not measured.

Conclusion

Isokinetic strength training resulted in greater improvement in pain and functional disability than sensory motor training or other conventional training for university football players with PTOA following ACL injury. The isokinetic training had a beneficial effect on levels of inflammatory biomarkers and negligible effect on levels of BMPs. Further research is warranted into the effects of isokinetic strength training in a range of sports injuries and types of sports.

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REFERENCES

1. Racine J, Aaron RK. Post-traumatic osteoarthritis after ACL injury. R I Med J 2014; 97: 25–38.
2. Dare D, Rodeo S. Mechanisms of post-traumatic osteoarthritis after ACL injury. Curr Rheumatol Rep 2014; 16: 448.
3. Palmieri-Smith RM, Thomas AC. A neuromuscular mechanism of post-traumatic osteoarthritis associated with ACL injury. *Exerc Sport Sci Rev* 2009; 37: 147–153.
4. Joy, Susan M. Osteoarthritis after anterior cruciate ligament injury: the quadriceps question. Exercise Sport Sci Rev 2009; 37: 112.
5. Amin S, Baker K, Niu J, Clancy M, Goggins J, Guermazi A, et al. Quadriceps strength and the risk of cartilage loss and symptoms progression in knee osteoarthritis. Arthritis Rheum 2009; 60: 189–198.
6. Knoop J, van der Leeden M, Roorda LD, Thorstensson CA, van der Esch M, Peter WF et al. Knee joint stabilization therapy in patients with osteoarthritis of the knee and knee instability: subgroup analysis in a randomized, controlled trial. J Rehabil Med 2014; 46: 703–707.
7. Myer GD, Ford KR, Barber Foss KD, Liu C, Nick TG, Hewett TE. The relationship of hamstrings and quadriceps strength to anterior cruciate ligament injury in female athletes. Clin J Sport Med 2009; 19: 3–8.
8. Yoon YS, Chai M, Shin DW. Football injuries at Asian tournaments. Am J Sports Med 2004; 32: 36–42.
9. Alentorn-Geli E, Myer GD, Silvers HJ, Samitier G, Romero D, Lazaro-Haro C et al. Prevention of non-contact anterior cruciate ligament injuries in soccer players. Part 1: Mechanisms of injury and underlying risk factors. Knee Surg Sports Traumatol Arthrosc 2009; 17: 705–729.
10. Laurens J. van Baardewijk, Ende J, Thunnissen S, Romiijn LM, Hawinkels LJ, Cier CFM et al. Circulating bone morphogenetic protein levels and delayed fracture healing. Int Orthopaed (SICOT) 2013; 37: 523–527.
11. Young WB, James R, Montgomery I. Is muscle power related to running speed with changes of direction? J Sports Med Phys Fitness 2002; 42: 282–288.
12. Brown TD, Johnston RC, Saltzman CL, Marsh JL, Buckwalter JA. Posttraumatic osteoarthritis: a first estimate of incidence, prevalence, and burden of disease. J Orthop Trauma 2006; 20: 739–744.
13. Nambi G, Abdelbasse WK, George J, Mansoor E. Radiological and Biochemical effects (CTX-II, MMP-3,8 and 13) of Ga-As Low Level Laser Therapy (LLLT) in chronic osteo arthritis in Al-Kharj, Saudi Arabia. Lasers Med Sci 2016; 32: 297–303.
14. Palmieri-Smith RM, Thomas AC, Wojtys EM. Maximizing quadriceps strength after ACL reconstruction. Clin Sports Med 2008; 27: 405–424.
15. Hiemstra LA, Webber S, MacDonald PB, Kriellaars DJ. Hamstring and quadriceps strength balance in normal and hamstring anterior cruciate ligament-reconstructed subjects. Clin J Sport Med 2004; 14: 274–280.
16. Riemann BL, Lephart SM. The sensorimotor system. Part I: The physiologic basis of functional joint stability. J Athlet Train 2002; 37: 71–79.
17. Carvalho N, Bittar S, Pinto F, Ferreira M, Sitta R. Manual for guided home exercises for osteoarthritis of the knee. Clinics 2010; 65: 775–780.
18. Ferraz MB, Quaresma MR, Aquino LR, Atra E, Tugwell P, Goldsmith CH, Reliability of pain scales in the assess-

ment of literature and illiterate patients with rheumatoid arthritis. J Rheumatol 1990; 17: 1022–1234.
19. Alghadir A, Anwer S, Iqbal ZA, Alsanawi HA. Cross-cultural adaptation, reliability and validity of the Arabic version of the reduced Western Ontario and McMaster Universities Osteoarthritis index in patients with knee osteoarthritis. Disabil Rehabil 2016; 38: 689–694.
20. Perry CD, Alekel DL, Ritland LM, Bhupathiraju SN, Stewart JW, Hanson LN. Centrally located body fat is related to inflammatory markers in healthy postmenopausal women. Menopause 2008; 15: 619–627.
21. Cheung RT, Smith AW, Wong del P. H:Q ratios and bilateral leg strength in college field and court sports players. J Hum Kinet 2012; 33: 63–71.
22. Eniseler N, Sahan C, Vurgun H, Mavi HF. Isokinetic strength responses to season-long training and competition in Turkish elite soccer players. J Hum Kinet 2012; 31: 159–168.
23. Ardern CL, Pizzari T, Wollin MR, Webster KE. Hamstrings strength imbalance in professional football (soccer) players in Australia. J Strength Cond Res 2015; 29: 997–1002.
24. Petersen J, Thorborg K, Nielsen MB, Jorgensen EB, Holmich P. Preventive effect of eccentric training on acute hamstring injuries in men's soccer: a cluster randomized controlled trial. Am J Sports Med 2011; 39: 2296–2303.
25. Menzel HJ, Chagas MH, Szmuchrowski LA, Araujo SR, Andrade AGP, Moraleide FR. Analysis of lower limb asymmetries by isokinetic and vertical jump tests in soccer players. J Strength Cond Res 2013; 27: 1370–1377.
26. Nickols-Richardson SM, Miller LE, Wootten DF, Ramp WK, Herbert WG. Concentric and eccentric isokinetic resistance training similarly increases muscular strength, fat-free soft tissue mass and specific bone mineral measurements in young women. Osteoporosis Int 2007; 18: 789–796.
27. Helmark IC, Mikkelsen UR, Borglum J, Rothe A, Petersen MC, Andersen O, et al. Exercise increases interleukin-10 levels both intra-articularly and peri-synovially in patients with knee osteoarthritis: a randomized controlled trial. Arthritis Res Ther 2010; 12: R126.
28. Wang Q, Rozelle A, Lepus C, Scanzello C, Song J, Larsen D, et al. Identification of a central role for complement in osteoarthritis. Nat Med 2011; 17: 1674–1679.
29. Riemann BL, Lephart SM. The sensorimotor system. Part II: The role of proprioception in motor control and functional joint stability. J Athlet Train 2002; 37: 80–84.
30. Aguiar GC, Do Nascimento MR, De Miranda AS, Rocha NP, Teixeira AL, Scalzo PL. Effects of an exercise therapy protocol on inflammatory markers, perception of pain, and physical performance in individuals with knee osteoarthritis. Rheumatol Int 2015; 35: 525–531.
31. Imamura M, Imamura ST, Kaziyama HHS, Targino RA, Hsing WT, de Souza LP, et al. Impact of nervous system hyperalgesia on pain, disability, and quality of life in patients with knee osteoarthritis: a controlled analysis. Arthrit Care Res 2008; 59: 1424–1431.