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http://dx.doi.org/10.1016/j.clinbiomech.2016.05.002

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Influence of a knee brace intervention on perceived pain and patellofemoral loading in recreational athletes.

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Keywords: Biomechanics, knee, brace, patellofemoral.

Word count: 3489
Abstract

**Background:** The current investigation aimed to investigate the effects of an intervention using knee bracing on pain symptoms and patellofemoral loading in male and female recreational athletes. **Methods:** Twenty participants (11 males & 9 females) with patellofemoral pain were provided with a knee brace which they wore for a period of 2 weeks. Lower extremity kinematics and patellofemoral loading were obtained during three sports specific tasks, jog, cut and single leg hop. In addition their self-reported knee pain scores were examined using the Knee injury and Osteoarthritis Outcome Score. Data were collected before and after wearing the knee brace for 2 weeks. **Findings:** Significant reductions were found in the run and cut movements for peak patellofemoral force/pressure and in all movements for the peak knee abduction moment when wearing the brace. Significant improvements were also shown for Knee injury and Osteoarthritis Outcome Score subscales symptoms (pre: male= 70.27, female= 73.22 & post: male= 85.64, female= 82.44), pain (pre: male= 72.36, female= 78.89 & post: male= 85.73, female= 84.20), sport (pre: male= 60.18, female= 59.33 & post: male= 80.91, female= 79.11), function and daily living (pre: male= 82.18, female= 86.00 & post: male= 88.91, female= 90.00) and quality of life (pre: male= 51.27, female= 54.89 & post: male= 69.36, female= 66.89). **Interpretation:** Male and female recreational athletes who suffer from patellofemoral pain can be advised to utilize knee bracing as a conservative method to reduce pain symptoms.

**Introduction**

Patellofemoral pain is the most common knee pathology (Dixit et al., 2007), characterized by retro-patellar pain mediated by prolonged sitting, stair climbing, and sports activities (Al-Hakim et al., 2012; Petersen et al., 2014). In athletic populations patellofemoral pain
symptoms force many to limit or even end their participation in sports activities (Blond & Hansen, 1998). Importantly it has been shown that between 71-91 % of those who present with patellofemoral pain have ongoing symptoms up to 20 years following diagnosis (Nimon et al., 1998). Furthermore, it has been suggested that patellofemoral pain may serve as a precursor to the progression of osteoarthritic symptoms in later life (Crossley 2014; Thomas et al., 2010). The prevalence of patellofemoral pain in athletic populations is considered to be between 8-40 %, with a greater frequency in females (Robinson and Nee, 2007; Boling et al., 2010). Although Selfe et al., (2016) found that in a patellofemoral subgroup with higher levels of physical activity 54% were males.

One of the functions of the patella as the body’s largest sesamoid bone is to enhance the effective moment arm of the quadriceps muscle group and reduce the mechanical effort required to extend the knee joint (Tumia and Maffulli, 2002). The articular surface of the patellofemoral joint is comprised of dense hyaline cartilage which is capable of bearing high, compressive loads (Garth, 2001). Patellofemoral contact forces are enhanced with increasing angles of knee flexion and can reach up to 8 B.W during sports tasks (Thomee et al., 1999).

Although the incidence of patellofemoral pain is high, the causative mechanisms which lead to the initiation of symptoms are not well understood. Those with patellofemoral pain are much more likely to be physically active than age-matched controls (Fulkerson, 2002). The current consensus is that there are multiple causative factors and that patellofemoral pain is the end result of numerous pathophysiological processes (Witvrouw et al., 2014). Aetiological research investigating the causes of patellofemoral symptoms has cited both extrinsic and intrinsic mechanisms as contributory factors. Extrinsic mechanisms consist of
overtraining, training errors and inferior athletic equipment (Tumia and Maffulli, 2002). Intrinsic biomechanical mechanisms consist of knee joint laxity, lower extremity mal-alignment and muscular imbalance (Tumia & Maffulli, 2002). In addition mechanical overloading of the patellofemoral joint is considered to be a key risk factor for the initiation of pain symptoms in athletes (LaBella, 2004; Ho et al., 2012). The knee abduction moment has also been shown to correspond with increased load borne by the lateral facet of the patellofemoral joint and thus also contribute to the aetiology of patellofemoral pain syndrome (Miyazaki et al., 2002; Zhao et al., 2007; Sigward et al., 2012; Myer et al., 2015). Excessive patellofemoral forces and knee abduction moments in conjunction with a high training volume leads to the initiation of symptoms, by overloading the patellofemoral joint beyond functional adaptive structural responses (LaBella, 2004; Dye, 2005; Ho et al., 2012).

Treatment options for patellofemoral pain typically include; exercise, patella taping, knee bracing, foot orthoses and manual therapy (Bolgla & Boling, 2010). Knee braces are defined as external, non-adhesive apparatus which attempt to alter the position of the patella (Paluska & McKeag, 2000). Knee braces come in a range of different interventions which typically include knee braces in a range of materials, sleeves and bandages (Bolgla & Boling, 2010). These are considered a relatively inexpensive treatment modality that can be purchased independently or prescribed by a therapist (Warden, 2008). Importantly the majority of knee braces can be applied by the wearer without assistance from a healthcare professional meaning that the user has more control over the management of their condition (Paluska & McKeag, 2000). A well-fitting knee orthosis can be used during normal daily activities and also during athletic pursuits (Warden 2008).
Although a substantial body of literature exists regarding the mechanical effects of knee bracing, there is currently a paucity of research investigating the influence of knee bracing for the treatment of symptoms in those with patellofemoral pain. Powers et al., (2004) showed that knee bracing provided an immediate improvement of 54% in knee pain symptoms which were assessed using a 10 cm visual analog scale. Arazpour et al., (2014) demonstrated that a 6 week intervention produced a significant reduction in knee pain symptoms. Khadavi & Fredericson (2015) showed that knee bracing produced significant reductions in the knee pain parameters which were examined via the Knee injury and Osteoarthritis Outcome Score (KOOS). Callaghan et al., (2015) found that knee bracing proved to be significantly better than control for reducing symptoms after a 6 week intervention, in patients with patellofemoral pain. Miller et al., (1997) however revealed that knee bracing produced only very small non-significant improvements in patellofemoral pain symptoms. Yu et al., (2015) similarly showed that neither tibiofemoral nor patellofemoral bracing provided any additional benefits in comparison to a control group which received no bracing.

To date there has been no published work which has examined the efficacy and effectiveness of knee bracing for the treatment of symptoms in recreational athletes with patellofemoral pain during sporting activities. Selfe et al., (2016) identified that different subgroups exist within the patellofemoral pain population and different treatments regimes may be more effective for each of the different subgroups. Selfe et al., (2016) showed that the ‘strong’ subgroup was characterized by higher levels of physical activity. Suggestions for the strong, more athletic subgroup included; proprioceptive training, taping and bracing although this has yet to be fully explored. Therefore the aim of the current investigation was to investigate the effects of an intervention using knee bracing on pain symptoms and patellofemoral loading in male and female recreational athletes. Research of this nature may improve understanding of
conservative management of patellofemoral pain and also provide recreational athletes with an alternative treatment. The current study tests the hypothesis that intervention using knee bracing will improve pain symptoms and reduce patellofemoral loading in recreational athletes with patellofemoral pain.

Methods

Participants

Twenty participants (11 male and 9 female) volunteered to take part in the current investigation. Participants were included into the study only if they showed symptoms of patellofemoral pain and no evidence of any other pathology. Patellofemoral pain diagnosis was made as a function of the clinical presentation of symptoms in accordance with the recommendations of Crossley et al., (2002). Participants were firstly required to exhibit symptoms of patellofemoral pain with no evidence of any other condition. The inclusion conditions were a) anterior knee pain resulting from two or more of the following; sustained sitting, climbing stairs, squatting, running, kneeling, and hopping or jumping; b) initiation of pain symptoms not caused by a specific painful incident; and c) manifestation of pain with palpation of the patellar facets. Participants were excluded from the study if there was evidence of any other knee pathology or had previously undergone surgery on the patellofemoral joint. In addition participants who had exhibited symptoms for less than 3 months or were taking any anti-inflammatory/ corticosteroid medications were also excluded. Finally participants who were aged 50 or above were excluded in order to reduce the likelihood of pain being caused by degenerative joint disease. Written informed consent was provided in accordance with the declaration of Helsinki. The procedure was approved by the
Universities Science, Technology, Engineering, Medicine and Health ethics committee, with the reference STEMH 295.

Knee brace

A single knee brace was used in this study, (Trizone, DJO USA), which came in three different sizes; small, medium and large to accommodate all participants (Figure 1).

Procedure

Participants were required to report to the laboratory on two occasions. On their initial visit to the laboratory they were required to complete five repetitions of three sports specific movements; jog, cut and single leg hop. In addition to this the participants also completed the KOOS questionnaire in order to assess self-reported knee pain. Once the biomechanical and KOOS data were obtained, participants were then provided with a knee brace in their size which they were asked to wear for all of their physical activities for 14 days. Participants were instructed to maintain their habitual sport/exercise regime and also recorded the number of hours spent exercising/ playing sport during the 14 days prior to the intervention and also during the intervention itself. Following the 14 day intervention participants returned to the laboratory where the protocol was repeated whilst wearing their knee brace.
Kinematic information from the lower extremity joints was obtained using an eight camera motion capture system (Qualisys Medical AB, Goteburg, Sweden) using a capture frequency of 250 Hz. Dynamic calibration of the system was performed before each data collection session. Calibrations producing residuals <0.85 mm and points above 4000 in all cameras were considered acceptable. To measure kinetic information an embedded piezoelectric force platform (Kistler National Instruments, Switzerland Model 9281CA) operating at 1000 Hz was utilized. The kinetic and kinematic information were synchronously obtained and interfaced using Qualisys track manager.

To quantify lower extremity joint kinematics in all three planes of rotation the calibrated anatomical systems technique was utilized (Cappozzo et al., 1995). Retroreflective markers (19 mm) were positioned unilaterally allowing the; foot, shank and thigh to be defined. The foot was defined via the 1st and 5th metatarsal heads, medial and lateral malleoli and tracked using the calcaneus, 1st metatarsal and 5th metatarsal heads. The shank was defined via the medial and lateral malleoli and medial and lateral femoral epicondyles and tracked using a cluster positioned onto the shank. The thigh was defined via the medial and lateral femoral epicondyles and the hip joint centre and tracked using a cluster positioned onto the thigh. To define the pelvis additional markers were positioned onto the anterior (ASIS) and posterior (PSIS) superior iliac spines and this segment was tracked using the same markers. The hip joint centre was determined using a regression equation that uses the positions of the ASIS markers (Sinclair et al., 2013). The centers of the ankle and knee joints were delineated as the mid-point between the malleoli and femoral epicondyle markers (Sinclair et al., 2015; Graydon et al., 2015). Each tracking cluster comprised four retroreflective markers mounted onto a thin sheath of lightweight carbon-fibre. Static calibration trials were obtained allowing for the anatomical markers to be referenced in relation to the tracking markers/clusters. The
Z (transverse) axis was oriented vertically from the distal segment end to the proximal segment end. The Y (coronal) axis was oriented in the segment from posterior to anterior. Finally, the X (sagittal) axis orientation was determined using the right hand rule and was oriented from medial to lateral. Data were collected during run, cut and hop movements according to below:

Run

Participants ran at 4.0 m.s$^{-1}$ ±5% and struck the force platform injured limb. The average velocity of running was monitored using infra-red timing gates (SmartSpeed Ltd UK). The stance phase of running was defined as the duration over > 20 N of vertical force was applied to the force platform (Sinclair et al., 2013).

Cut

Participants completed 45° sideways cut movements using an approach velocity of 4.0 m.s$^{-1}$ ±5% striking the force platform with their injured limb. Cut angles were measured from the centre of the force plate and the corresponding line of movement was delineated using masking tape so that it was clearly evident to participants (Sinclair et al., 2015). The stance phase of the cut-movement was similarly defined as the duration over > 20 N of vertical force was applied to the force platform (Sinclair et al., 2013).

Hop
Participants began standing by on their injured limb; they were then requested to hop forward maximally, landing on the force platform with same leg without losing balance. The arms were held across the chest to remove arm-swing contribution. The hop movement was defined as the duration from foot contact (defined as > 20 N of vertical force applied to the force platform) to maximum knee flexion. The hop distance was recorded in the initial data collection session as was maintained for the second testing session.

Data processing

Dynamic trials were processed using Qualisys Track Manager and then exported as C3D files. GRF and marker data were filtered at 50 Hz and 15 Hz respectively using a low-pass Butterworth 4th order filter and processed using Visual 3-D (C-Motion, Germantown, MD, USA). Joint kinetics were computed using Newton-Euler inverse-dynamics, allowing net knee joint moments to be calculated. Angular kinematics of the lower extremity joints were calculated using an XYZ (sagittal, coronal and transverse) sequence of rotations. To quantify joint moments segment mass, segment length, GRF and angular kinematics were utilized using the procedure previously described by Sinclair, (2014). The net joint moments were normalized by dividing by body mass (Nm/kg). Discrete lower extremity joint kinematic measures were extracted for statistical analysis were 1) peak angle and 2) relative range of motion (representing the angular displacement from footstrike to peak angle).

Knee loading was examined through extraction of peak knee abduction moments, patellofemoral contact force (PTCF) and patellofemoral contact pressure (PTS). PTCF was normalized by dividing the net PTCF by body weight (B.W). PTCF loading rate (B.W/s) was
calculated as a function of the change in PTCF from initial contact to peak force divided by the time to peak force.

PTCF during running was estimated using knee flexion angle (kf) and knee extensor moment (KEM) through the biomechanical model of Ho et al., (2012). This model has been utilized previously to resolve differences in PTCF and PTS in different footwear (Bonacci et al., 2013; Kulmala et al., 2013; Sinclair, 2014) and between those with and without patellofemoral pain (Keino & Powers, 2002). The model has also been shown to be sufficiently sensitive to detect differences in PTCF between sexes (Sinclair and Bottoms, 2015).

The effective moment arm distance (m) of the quadriceps muscle (QM) was calculated as a function of kf using a non-linear equation, based on information presented by van Eijden et al., (1986):

\[ QM = 0.00008 \, kf^3 - 0.013 \, kf^2 + 0.28 \, kf + 0.046 \]

The force (N) of the quadriceps (FQ) was calculated using the below formula:

\[ FQ = \frac{KEM}{QM} \]

Net PTCF (N) was estimated using the FQ and a constant (C):
PTCF = FQ * C

C was described in relation to kf using a curve fitting technique based on the non-linear equation described by van Eijden et al., (1986):

\[ C = \frac{0.462 + 0.00147 \times kf^2 - 0.0000384 \times kf^2}{1 - 0.0162 \times kf + 0.000155 \times kf^2 - 0.000000698 \times kf^3} \]

PTS (MPa) was calculated using the net PTCF divided by the patellofemoral contact area. The contact area was described using the Ho et al., (2012) recommendations by fitting a 2nd order polynomial curve to the data of Powers et al., (1998) showing patellofemoral contact areas at varying levels of kf.

PTS = PTCF / contact area

Statistical analyses

Descriptive statistics of means and standard deviations were obtained for each outcome measure. Shapiro-Wilk tests were used to screen the data for normality. Differences in biomechanical and knee pain parameters were examined using 2 (BRACE) x 2 (GENDER) mixed ANOVA’s. Differences in physical activity duration prior to and during the intervention were examined using a paired samples t-test. Statistical significance was
accepted at the p<0.05 level (Sinclair et al., 2013). Effect sizes for all significant findings were calculated using partial Eta$^2$ ($p\eta^2$). All statistical actions were conducted using SPSS v22.0 (SPSS Inc, Chicago, USA). In accordance with the recommendations of Roose & Lohmander, (2003) minimal perceptible clinical improvements (MCIP) were considered to be 10 points on each of the KOOS subsections.

**Results**

Tables 1-4 present the knee pain and patellofemoral variables obtained before and after the knee brace intervention. The results show that both knee pain and patellofemoral loading were significantly influenced by the intervention using knee bracing.

**Physical activity duration**

No significant differences (P>0.05) in physical activity duration were observed, participants completed mean 4.40 and SD 2.11 hours of physical activity/sport prior to the intervention and mean 4.37 and SD 2.32 during.

**Knee pain**

For the KOOS symptoms (P<0.05, $p\eta^2 = 0.71$) and pain (P<0.05, $p\eta^2 = 0.71$) subsections significant improvements were observed following the intervention, with 16 of the 20 participants demonstrating improvements. For the KOOS function and daily living (P<0.05, $p\eta^2 = 0.65$) and sports (P<0.05, $p\eta^2 = 0.66$) subsections significant improvements were found following the intervention, with 17 and 18 of the 20 participants demonstrating improvements.
respectively. Finally for the quality of life subsection a significant improvement ($P<0.05$, $\eta^2 = 0.28$) was found as a function of the intervention, with 16 of the 20 participants demonstrating improvements (Table 1).

### Table 1 near here

#### Patellofemoral kinetics

**Run**

For both PTCF ($P<0.05$, $\eta^2 = 0.27$) and PTS ($P<0.05$, $\eta^2 = 0.24$) there were significant reductions following the intervention. For PTCF loading rate there was also a significant ($P<0.05$, $\eta^2 = 0.39$) reduction following the intervention. Finally, there was a significant ($P<0.05$, $\eta^2 = 0.25$) reduction in the peak knee abduction moment following the intervention (Table 2).

### Table 2 near here

#### Cut

For both PTCF ($P<0.05$, $\eta^2 = 0.29$) and PTS ($P<0.05$, $\eta^2 = 0.25$) there were significant reductions following the intervention. For PTCF loading rate there was also a significant ($P<0.05$, $\eta^2 = 0.30$) reduction following the intervention. Finally, there was a significant ($P<0.05$, $\eta^2 = 0.23$) reduction in the peak knee abduction moment following the intervention (Table 3).
There was a significant (P<0.05, $\eta^2 = 0.27$) reduction in the peak knee abduction moment following the intervention (Table 4).

For peak hip flexion there was a significant (P<0.05, $\eta^2 = 0.34$) reduction following the intervention. Similarly for peak knee flexion there was a significant (P<0.05, $\eta^2 = 0.35$) reduction following the intervention.

For peak hip flexion there was a significant (P<0.05, $\eta^2 = 0.32$) reduction following the intervention. Similarly for peak knee flexion there was a significant (P<0.05, $\eta^2 = 0.34$) reduction following the intervention.
For peak hip flexion there was a significant (P<0.05, $\eta^2 = 0.33$) reduction following the intervention. Similarly for peak knee flexion there was a significant (P<0.05, $\eta^2 = 0.36$) reduction following the intervention.

**Discussion**

The aim of the current investigation was to determine the biomechanical efficacy and clinical effectiveness of knee bracing in recreational athletes with patellofemoral pain. To the authors' knowledge this represents the first investigation to examine the effects of knee bracing on recreational athletic participants suffering from patellofemoral pain. Given the high incidence of patellofemoral pain in recreational athletes, research of this nature may provide important clinical information regarding the conservative management of patellofemoral pain.

The first key observation from the current work supports our hypothesis in that knee bracing served to significantly reduce all of the participant reported indicators of knee pain. The magnitude of the improvements in all subsection of the KOOS questionnaire exceeded the minimum threshold required for clinical relevance (Roose & Lohmander, 2003). This in conjunction with the observation that the majority of participants (N=≥16/20) exhibited improvements in symptoms is a key clinical finding. Importantly, this work also showed that activity duration did not differ, meaning that improvements in pain symptoms did not appear to be mediated through reductions in physical activity. This indicates that knee bracing has the potential to provide clinically meaningful improvements in patient reported symptoms in recreational athletes with patellofemoral pain.
It is proposed that the improvements in patient reported symptoms were mediated through reductions in PTCF and PTS which were observed following the brace intervention. This observation is similarly in support of our hypothesis and it is proposed that it relates to the reduction in the magnitude of peak knee flexion found in the brace condition. Reduced knee flexion serves to attenuate the knee extensor moment requirement during landing tasks, thus the loads imposed on the patellofemoral joint are reduced (Thomee et al., 1999). It is unknown whether this observation relates to restriction about the knee joint imposed by the brace which would be undesirable for athletes where full range of movement is required. Future work should therefore focus on the proprioceptive and potential restrictive effects of these braces.

In addition reduced knee abduction moments were also observed as a function of the brace intervention. This finding may also have clinical relevance given the relation between knee abduction moment and the aetiology of patellofemoral pain. As such reductions in the magnitude of the knee abduction moment may be a further mechanism by which knee bracing served to improve patellofemoral pain symptoms. Knee bracing aims to reduce the magnitude of the abduction moment created by the ground reaction force by brace applying a constant moment about the knee (Pagini et al., 2010). Therefore it is proposed that this finding relates to the mechanical influence of the knee brace itself.

A potential drawback of the current investigation is that patellofemoral loading was quantified using a musculoskeletal modelling approach. This technique was necessary as direct quantification of patellofemoral forces necessitate the utilization invasive measurement techniques, which are not possible due to ethical considerations. Regardless, the utilization of
the knee extensor moment as the primary input measurement into the calculation of patellofemoral loading means that antagonist forces that act in the opposite direction of the joint remain unaccounted for (Sinclair & Bottoms, 2015). Therefore this may lead to an underestimation patellofemoral loading during the dynamic activities (Sinclair & Selfe, 2015). A further potential limitation of the current work is the lack of a control group. Whist the current study observed improvements in self-reported pain as a function of the intervention despite no change in activity, the lack of a control group means the possibility that improvements were caused by a factors other than those measured here cannot be ruled out. Future clinical research may wish to investigate the effects of knee bracing in patellofemoral pain in recreational athletes using a randomized controlled research design.

In conclusion, although previous analyses have investigated the effects of knee bracing, the current knowledge with regards to the effects of bracing in recreational athletes with patellofemoral pain is limited. Recreational athletes represent a significant proportion of patellofemoral pain patients, thus research of this nature may provide important clinical information. The current investigation therefore addresses this firstly by providing a comparison of knee pain symptoms before and after an intervention using knee bracing and secondly by contrasting the biomechanics of different sports movements before and after the intervention. In addition this study shows significantly improvements in patient reported symptoms and significantly reductions in knee loading following the intervention. The key implication from this study is that male and female recreational athletes who suffer from patellofemoral pain may be advised that utilizing knee bracing as a conservative management can reduce pain symptoms.


Potential reviewers

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Table 1: Knee pain symptoms as a function of both knee brace intervention and gender.

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<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>Brace</td>
<td>No-brace</td>
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<tr>
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<td>SD</td>
<td>Mean</td>
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Table 2: Patellofemoral kinetics during running as a function of both knee brace intervention and gender.

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<tr>
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<tr>
<td><strong>PTCF (B.W)</strong></td>
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<td><strong>PTS (MPa)</strong></td>
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<td><strong>PTCF loading rate (B.W/s)</strong></td>
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<td><strong>Peak abduction moment (Nm/kg)</strong></td>
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Table 3: Patellofemoral kinetics during cutting as a function of both knee brace intervention and gender.

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Table 4: Patellofemoral kinetics during the single leg hop as a function of both knee brace intervention and gender.

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Please mark ‘X’ or ‘√’ in the ‘Tick’ column to verify that the manuscript has met the requirements needed prior to review.

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<tr>
<th>Basic requirements</th>
<th>Author response or further detail</th>
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<tbody>
<tr>
<td>Highlights</td>
<td>Avoid using abbreviations in highlights and ensure each bullet point (between 3 &amp; 5 required) doesn't exceed 85 characters</td>
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<td>Title page</td>
<td>Avoid using abbreviations in the title</td>
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<td>Title page</td>
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<td>Word Count</td>
<td>Give word counts on the title page for both the abstract and the main text (excluding references and legends). The length should not normally exceed 4000 words with around six figures/tables (large data tables and multi-part figures are generally best placed in Supplementary Data).</td>
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<td>Abstract</td>
<td>In the Abstract, the following section headings (in italics) should each start on a new line: Background, Methods, Findings, and Interpretation. Only universally accepted and understood abbreviations are allowed in the Abstract (e.g. CT, MR), but no specialties or author-defined abbreviations (e.g. OA, osteoarthritis; TKR, total knee replacement etc). Please also ensure bullet points DO NOT appear in the abstracts. Finally the abstract must not exceed 250 words.</td>
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| Standard Deviation       | Avoid using ± symbol (in-text & tables) - use for example "mean xx (SD yy)"  
The abbreviation "SD" (standard deviation) should be set without the dots | X    |
| General                  | Add country to all addresses                                                                      | X    |
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| Acronyms                 | Acronyms need to be defined at first use.                                                        | X    |
| Acronyms                 | Acronyms with 'of' in them such as ‘range of motion’ should be abbreviated as RoM (not ROM)       | X    |
| Equipment info           | Sources of equipment etc. should have company, city, and country.                                | X    |
| Section heading          | The main text should be divided into appropriate headings: Introduction, Methods, Results, Discussion, and Conclusions.  
Section 2 of main text should be Methods (not Material and Methods)  
Subheadings may also be used and reviews may use other headings. | X    |
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